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RELATIONSHIP BETWEEN BLOOD GROUP GENES AND ECONOMIC TRAITS OF POULTRY

A THESIS

SURMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

by

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EDMONTON, ALBERTA
JULY, 1961



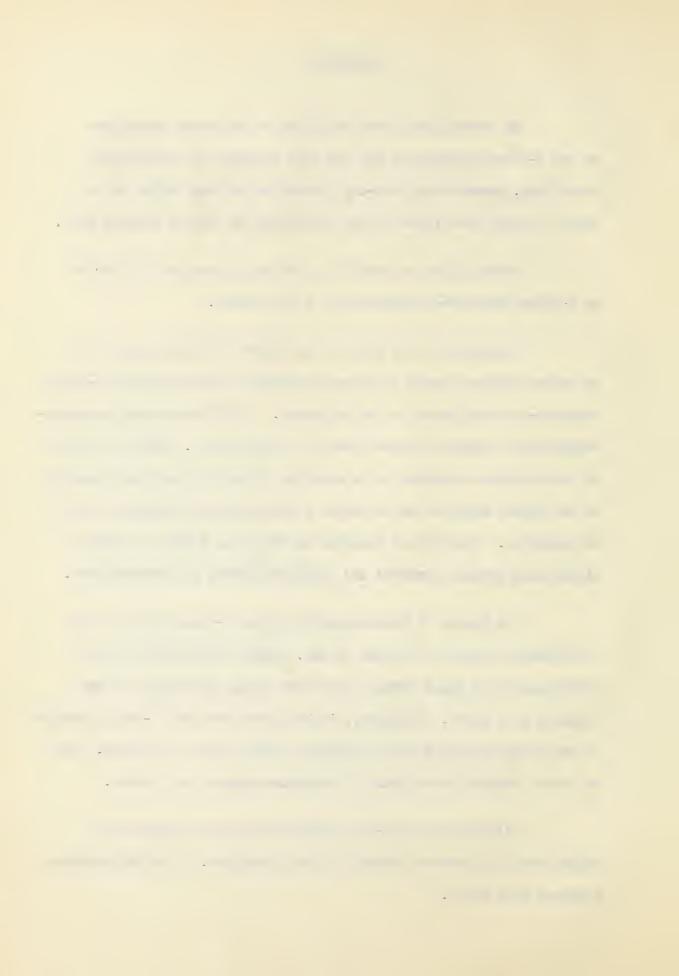
An investigation was initiated to determine the effect of the B-locus genotype of the red cell antigens on hatchability, livability, growth rate, hen-day production and egg weight in the White Plymouth Rock flock at the University of Alberta Poultry Farm.

Hatchability in this flock did not appear to be affected by B-locus expected-heterozygosity of the embryo.

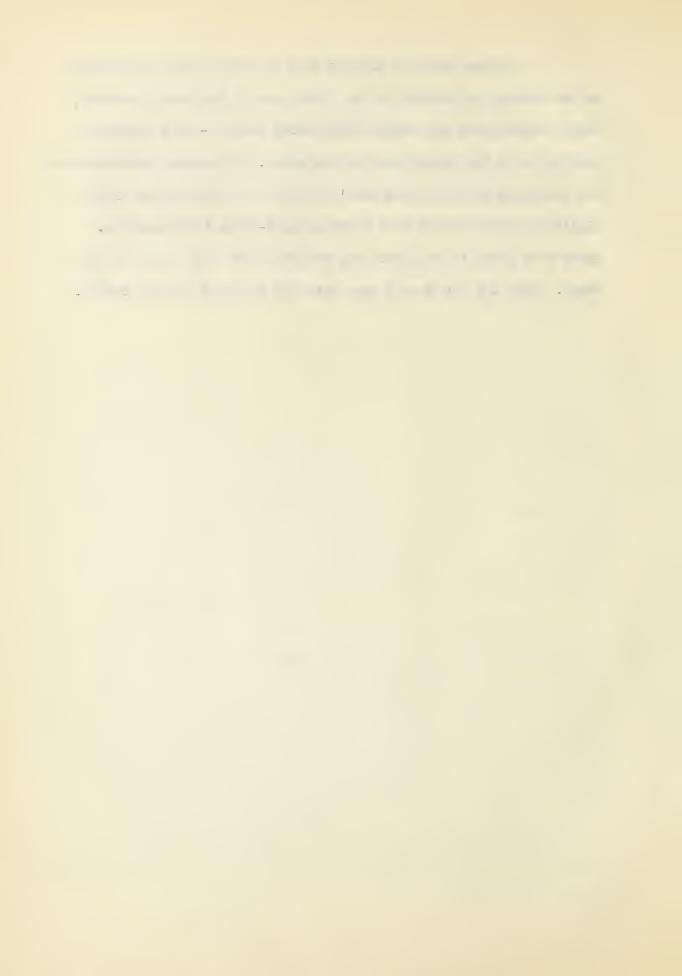
Invability to 10 weeks of age based on fertile eggs and on chicks hatched seemed to increase slightly with increasing B-locus expected-heterozygosity in the offspring. Differences among expected-heterozygous classes, however, were not significant. When the number of heterozygotes observed to be alive at 10 weeks of age was compared to the number expected to be alive, a heterozygous advantage seemed to be indicated. Statistical analysis of the data, however, showed no differences between observed and expected numbers of heterozygotes.

The degree of heterozygosity at the B-locus did not seem to influence growth to 6 weeks of age. Highly significant weight differences were found between sexes and among the progeny of dam families at 6 weeks. Similarly, it was found that the B-locus genotype of the chick did not seem to influence growth rate to 10 weeks. Sex and sire, however, were found to influence growth to 10 weeks.

Adjusted mean hen-day productions were not found to be significantly different between B-locus genotypes. Sire differences, however, were noted.



B-locus genotype did not seem to have any real influence on the average egg weight in the first year of the study, however, highly significant egg weight differences among B-locus genotypes were noted in the second year of the study. No general overdominance was indicated in the second year's data for egg weight but highly significant differences were found among B-locus heterozygotes. Sires were found to influence egg weight in the first year of the study. Data for the second year were not analyzed in this regard.



ACKNOWLEDGEMENTS

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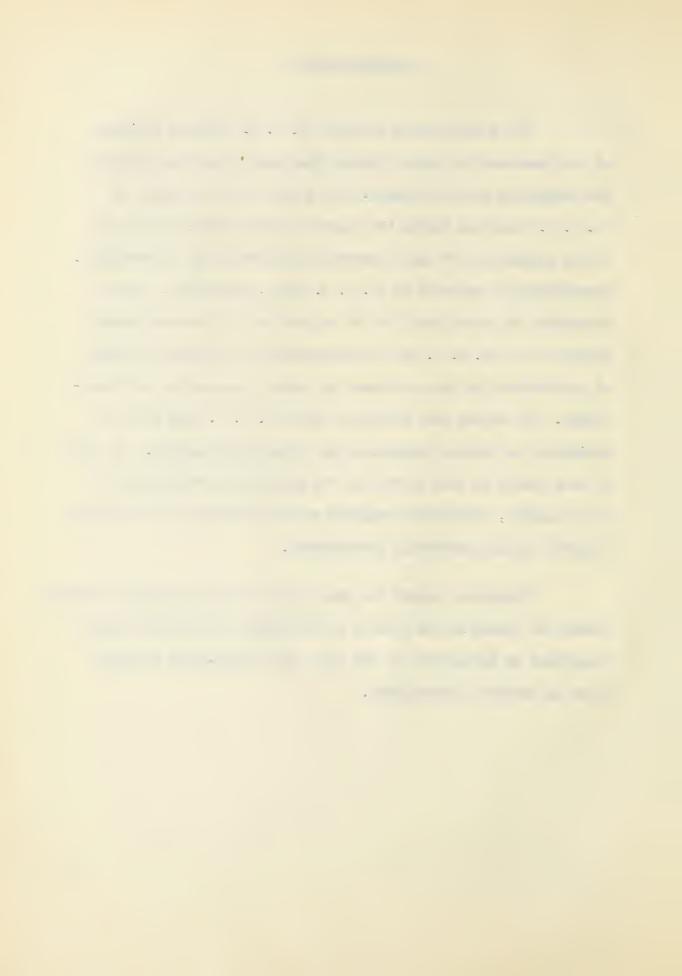


TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	2
Early History	2
Inheritance of Red Cell Antigens	Į.
Inbreeding and Blood Genotype Heterozygosity	7
Economic Significance of Blood Group Alleles	8
STUDIES AT THE UNIVERSITY OF ALBERTA	12
Experimental (General)	12
Sources of Data	12
Blood-typing	13
Experiment I. The Effect of B-locus Genotype of the Red Cell Antigens on Hatchability	13
Object	13
Experimental	114
Results and Discussion	16
Summary	18
Experiment II. The Effect of B-locus Genotype of the Red Cell Antigens on Livability to Ten Weeks	18
Object	18
Experimental	18
Results and Discussion	20
Summary	22
Experiment III. The Effect of the B-locus Genotype of the Red Cell Antigens on Growth Rate	23
Object	23

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	Page
Experimental	23
Results and Discussion	25
Summary	30
Experiment IV. The Effect of the B-locus Genotype of the Red Cell Antigens on Hen-Day Production	31
Object	31
Experimental	31
Results and Discussion	32
Summary	34
Experiment V. The Effect of the B-locus Genotype of the Red Cell Antigens on Egg Weight	34
Object	34
Experimental	34
Results and Discussion	35
Summary	39
GENERAL SUMMARY	40
I. The Effect of B-locus Genotype of the Red Cell Antigens on Hatchability	40
II. The Effect of B-locus Genotype of the Red Cell Antigens on Livability to Ten Weeks	40
III. The Effect of B-locus Genotype of the Red Cell Antigens on Growth Rate	41
IV. The Effect of the B-locus Genotype of the Red Cell Antigens on Hen-Day Production	42
V. The Effect of the B-locus Genotype of the Red Cell Antigens on Egg Weight	42
BIBLIOGRAPHY	11/4

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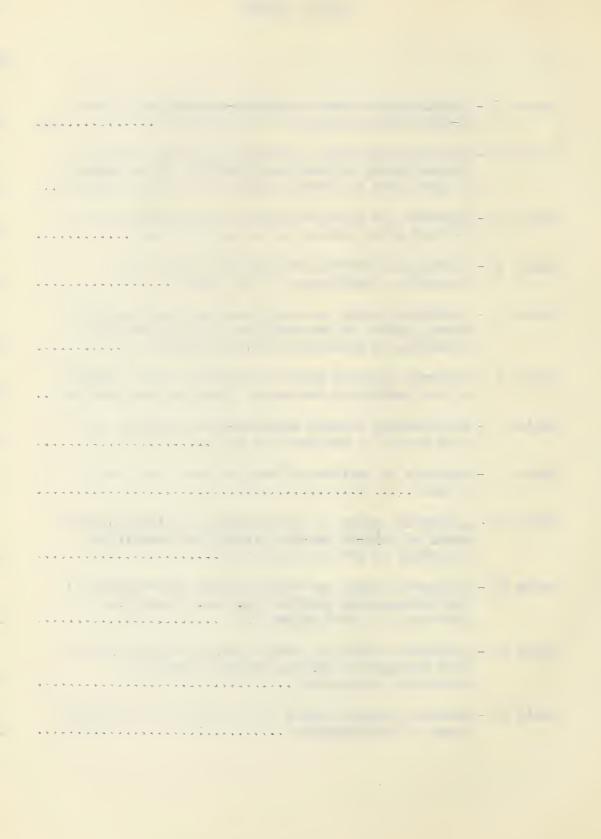
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	Page
APPENDIX A	47
Collection and Processing of Donor Blood	47
Immunization	47
Titer Test	48
Preparation of Antisera	49
Classification of Antisera into Reagents	49
B-locus Typing	49
APPENDIX B	50
Analysis of Ten Week Chick Weights	50
Analysis of Egg Production and Average Egg Weight	52

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LIST OF TABLES

				Page
Table	1	-	Relationship between expected-heterozygosity for B-blood group alleles and hatchability	16
Table	2	-	Relationship between expected-heterozygosity for B-blood group alleles and livability to ten weeks of age based on fertile eggs and on chicks hatched	20
Table	3	-	Observed and expected number of heterozygotes for B-blood group alleles at ten weeks of age	22
Table	4	-	Correlation between ten-week body weight and inbreeding coefficient of the chicks	25
Table	5	-	Arithmetic means and corresponding ranked adjusted means (grams) of ten-week body weight classified according to genotype, sire, hatch and sex	26
Table	6	**	Observed adjusted means and expected means (grams) of body weight at ten weeks of age in heterozygotes	28
Table	7	-	Relationship between expected-heterozygosity and body weight at six weeks of age	29
Table	8	-	Analysis of variance of body weight at six weeks of age	29
Table	9	•••	Arithmetic means and corresponding ranked adjusted means of 1959-60 hen-day productions classified according to genotype and sire	33
Table	10	-	Arithmetic means and corresponding ranked adjusted 1959 average egg weights (oz./doz.) classified according to genotype and sire	36
Table	11	***	Arithmetic means and corresponding ranked adjusted 1960 average egg weights (grams) classified according to genotype	37
Table	12	-	Observed adjusted means of egg weight and expected means in heterozygotes	38

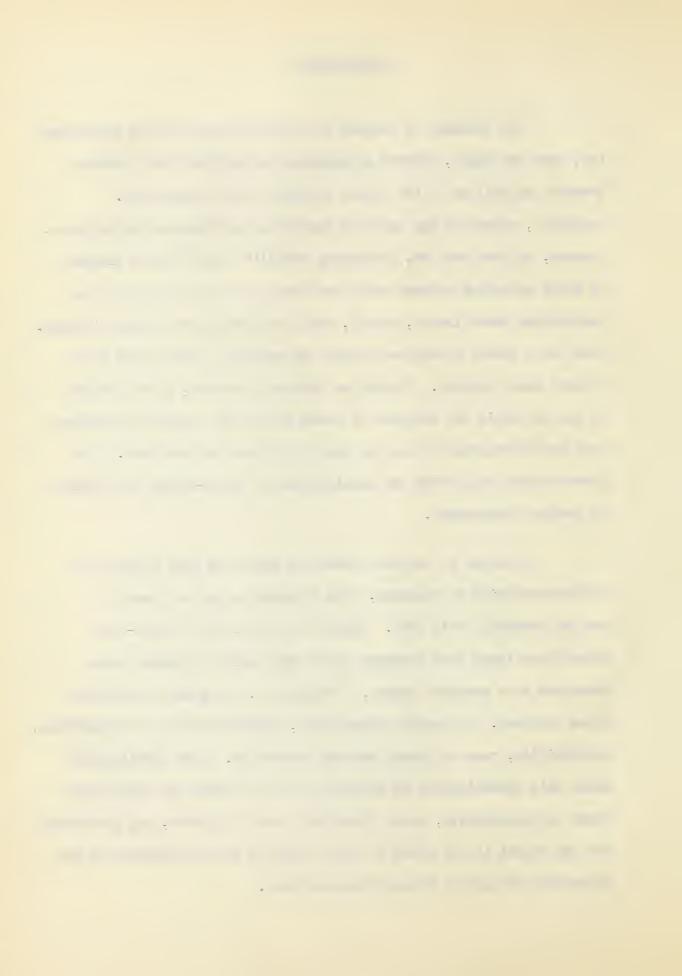


INTRODUCTION

The breeding of poultry for improved egg and meat production has, over the years, offered a challenge to the "would be" poultry breeder, as well as to the highly trained poultry geneticist.

Initially, selection was based on individual performance and pedigree. However, as time went on, increasing mortality rates forced breeders to adopt selection methods which had been shown to be effective in controlling death losses, namely, family selection and progeny testing. Under this latter selection program the mortality problem was soon brought under control. Production advances, however, were slow and it was not until the adoption of breed and strain crossing techniques that marked progress in egg and meat production was achieved. The present study deals with the significance of blood-typing in relation to poultry improvement.

Although it has been known for some time that blood group differences exist in chickens, this information was not used in poultry breeding until 1948. Workers in the field of blood-type inheritance found that antigens on the red cells of chickens were inherited in a specific manner. Furthermore, the genes controlling these antigens, or closely linked genes, seemed to influence livability, hatchability, rate of growth and egg production. More specifically then, this investigation is concerned with the effect of blood group genes on hatchability, chick livability, rate of growth, egg production, and egg weight in the flock of White Plymouth Rocks maintained at the University of Alberta Poultry Research Farm.



REVIEW OF LITERATURE

Early History

Red cell antigenic differences were first discovered in 1900 by Landsteiner, who elucidated the A-B-O blood group system of man and by Ehrlich and Morgenroth who demonstrated antigenic differences in goats. The first differences in chicken red cell antigens were discovered in 1924 by Landsteiner and Miller. They found eight different red cell types among ten chickens by using rabbit anti-chicken sera.

Todd (1930a) worked with iso-agglutinating sera produced mainly in Plymouth Rocks and found that if a polyvalent serum were exhausted with the cells of the father, cells from 17 per cent of the offspring were negative to the exhausted serum and if the polyvalent serum were exhausted with cells of the mother, a different 17 per cent of the progeny were negative. If the polyvalent serum were absorbed with cells from both parents, the red corpuscles of the offspring were completely non-reactive to the exhausted serum. Todd proposed therefore that the red cells of the progeny have receptors which are possessed by at least one of the parents. In later work, Todd (1930b) indicated that the antigenic units of the corpuscles maintained their independence during hereditary transmission, as evidenced by family agglutination patterns. Further evidence for the heritability of factors on the red corpuscles of chickens was reported by Todd (1935). He noted that the red cells of an inbred population exhibited a high degree of similarity as contrasted with varying degrees of dissimilarity observed between red cells of progeny from unrelated fowls. Kozelka (1933) obtained



similar results with hetero-agglutinating serum produced by the injection of chicken red corpuscles into rabbits. He concluded that the factors concerned with hemagglutination were found in various breeds of chickens but that these factors were distributed differently in various individuals.

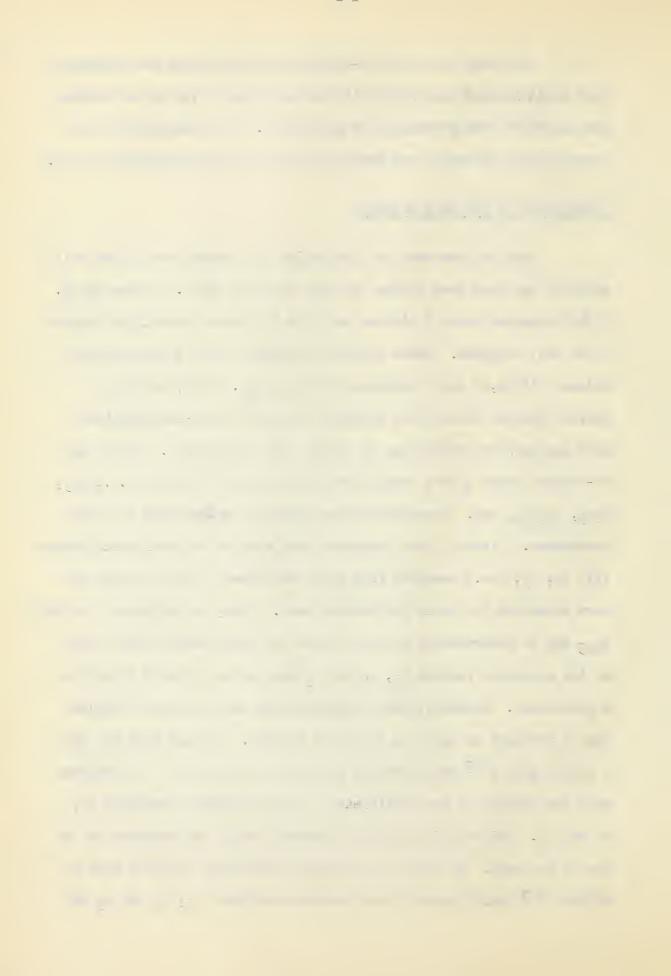
Little work had been done to relate chicken red cell antigens to an allele type of inheritance until Wiener (1934) re-analyzed Todd's results (1930b). Wiener stated that the agglutingens of fowl blood were inherited as simple Mendelian dominants and thus no chick possesses an agglutinogen not already present in the blood of one or both parents. In contrast to these conclusions, Thomsen (1934, 1936) and Boyd and Alley (1940) gave evidence indicating that red cell antigens may be the results of recessive genes or the complementary effect of nonallelic genes. Instances were cited in which the red cells of an individual reacted with an anti-serum while the red cells of either parent would not react. Subsequent work by others failed to confirm these results. A type of dominant and recessive inheritance was hypothesized by Olson (1943). By means of cross-absorptions and agglutination tests using normal bovine serum on the cells of Rhode Island Red full sibs, he found three main types of cells which he designated, I, II, and III. The type I cell possessed much more agglutinogen than type III while type II had none of the substance. Therefore the genotypes AA for type I, Aa for type III and aa for type II cells were proposed. Later work by Briles et al. (1952) also indicated that certain agglutinogens exhibited a dosage effect.



The work of the afore-mentioned investigators has indicated that agglutinogens associated with the red blood cells of the chicken are inherited from generation to generation. It remained for later investigators to expand and define the type of inheritance more fully.

Inheritance of Red Cell Antigens

Most of the work in elucidating the inheritance of red cell antigens has been done during the past thirteen years. Briles et al. (1948) reported seven B alleles and five D alleles controlling chicken blood cell antigens. These alleles, now named A and B respectively, Gilmour (1960a,b) were studied by Briles et al. (1950) mostly in Barred Plymouth Rocks using reagents prepared by iso-immunizations with appropriate absorptions to obtain high specificity. Briles and co-workers found a very complex series of A agglutinogens, e.g., A23, A123, A23156, etc. They offered two possible explanations for this phenomenon. Firstly, they suggested that each of the antigenic factors (A1, A2, A3, etc.) resulted from genes which were closely linked and were inherited in fairly predictable sets. Thus, an "antigenic complex" A123 may be inherited as a unit because the genes which produce each of the antigenic factors A1, A2 and A3 may be very closely linked on a chromosome. Secondly, they suggested that each antigenic complex may be produced by one of a series of alleles. If such were the case a single gene A¹²³ would produce an antigenic substance or substances with the ability to react with each of the serological reagents Al, A2 and A3. This multiple allele hypothesis could be accounted for in one of two ways. An allele may exhibit pleiotropy, in which case an allele A123 could produce three separate antigens, A1, A2 and A3 and



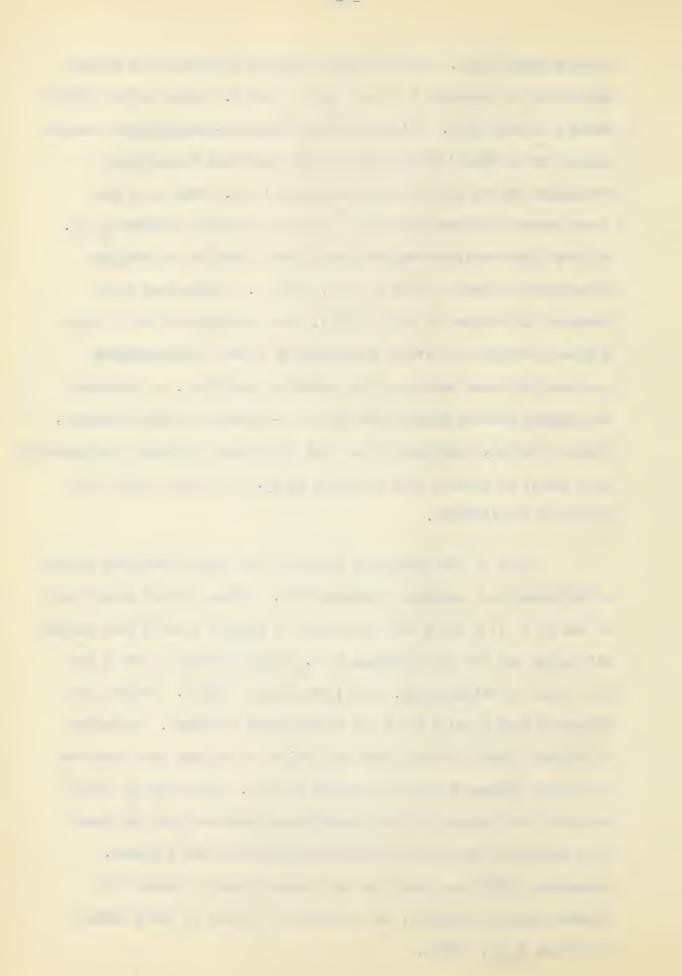
each of the latter would react with a different reagent. On the other hand an allele may produce an antigenic substance very similar to. yet chemically distinct from, antigenic substances produced by other members of the same allelic series. Thus, a reagent produced against an antigen which resulted from one member of an allelic series may cross-react with antigens produced by other members of the same allelic series. Briles et al. (1950) favoured the multiple allele hypothesis. Their results also indicated a complete lack of dominance because each allele seemed to produce its antigenic substance regardless of the other alleles present. Later work by Briles et al. (1959) tended to verify these conclusions. Gilmour (1959) found nine B alleles among four highly inbred White Leghorn lines of widely different origins. Absorption work by this investigator led him to believe that these antigens were quite distinct. Gilmour pointed out that the crossreactivities, which he and Briles et al. (1957) have found, do not fall into a definite pattern. Briles et al. and Gilmour (as reported by Gilmour, 1959) hypothesized that the B-locus is made up of a large number of multiple alleles each of which determines one antigen but that the individual antigens are able to "cross-react". These crossreactivities may result because each antigen consists of a large number of antigenic factors which will eventually be identified by ordinary serological methods.

The identification of autosomal loci determining red cell antigens was carried out chiefly by Briles and Gilmour. Briles et al. (1950) reported nine alleles at the A locus and five at the B locus (previously referred to by Briles et al. (1948) as B and D

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locus respectively). In 1951 Briles reported that the blood groups determined at autosomal loci were A, B, C and D. Later Briles (1956b) added E to this list. Gilmour (1960a) using iso-immunizations between members of an inbred White Leghorn line discovered two antigens determined by two alleles at an autosomal locus. This locus was later shown to correspond to the A locus postulated by Briles et al. Gilmour later developed reagents which were specific for antigens determined by genes at loci B, L, E, N and C. This worker also observed differences in antigenicity, since antigens of the A, B and E types produced very strong sera while L, N and C type antigens produced only weak antisera after repeated injections. An exchange of reagents between Gilmour and Allen (co-worker of Briles) revealed, Gilmour (1960a), that four of the loci discovered by Gilmour corresponded with the A, B, C and E loci of Briles et al. while the L and N loci proved to be different.

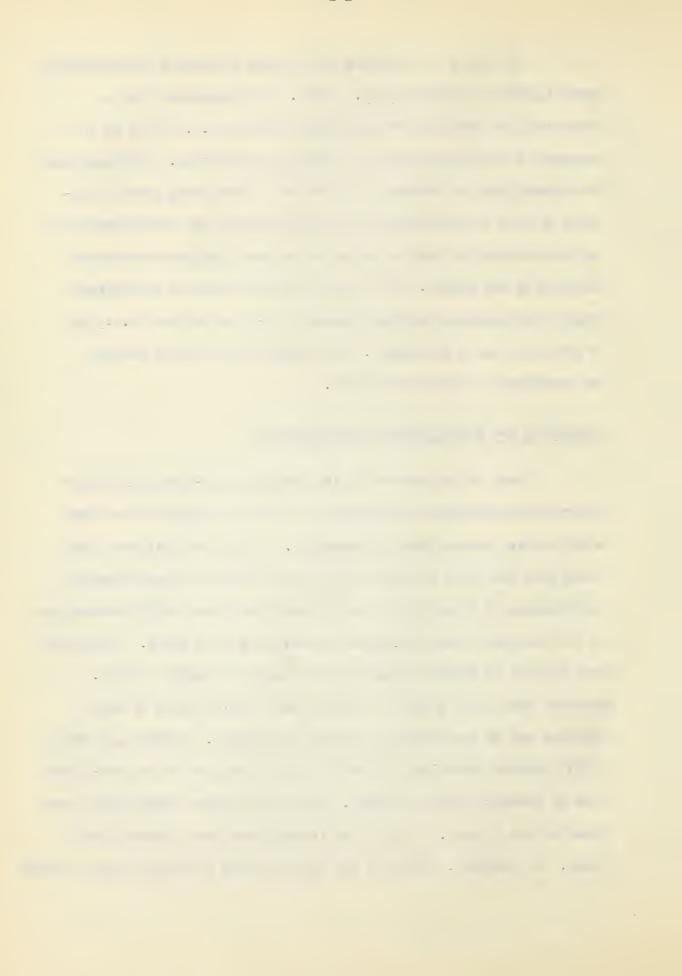
Most of the literature indicates that the blood group alleles at different loci segregate independently. Briles (1956a) showed that of the A, B, C, D and E loci with which he worked, A and E were linked with about one per cent crossing over. Linkage between A and E was also found by Briles et al. (1959) and Gilmour (1959). Briles also suggested that A and D and D and C loci might be linked. According to Gilmour (1960a) further data led Briles to conclude that there was no linkage between A and D or between D and C. Briles et al. (1959) reported that linkage has been established between a gene for crest (Cr) and X3, an antigenic substance apparently of the A system. Scheinberg (1956) has described two linkage groups of genes for chicken cellular antigens, one of which may belong to the A system of Briles et al. (1950).



The terms of reference with regard to poultry immunogenetics were clarified by Briles et al. (1957). They suggested that a superscript or subscript be used with a letter, i.e., B⁶ or B₆ to designate a particular allele or antigen respectively. They abandoned the concept that an antigenic product of a blood group gene is composed of more or less discrete antigenic factors and recommended that the term antigen be used to refer to the total antigenic substance produced by one allele. They further suggested that a serological reagent containing an antibody against a B-locus antigen, e.g., B₆ be designated as a B₆ reagent. This terminology will be used in the remainder of this dissertation.

Inbreeding and Blood Genotype Heterozygosity

Many of the workers in the poultry blood-typing field have observed considerable heterozygosity at several antigenic loci even after several generations of inbreeding. Briles and McGibbon (1948) found that two lines of Single Comb White Leghorns having inbreeding coefficients of 52 and 60 per cent respectively were still heterozygous at the loci which they designated at that time as B and D. Indications that alleles do change frequency were reported by Briles (1952). It appeared that one B allele in each of three inbred lines of White Leghorns may be lost through continued inbreeding. Schultz and Briles (1953) studied three loci in twelve inbred lines and in one non-inbred line of production bred chickens. Six of the twelve inbred lines were fixed at the D locus. Nine of the inbred lines were fixed at the D

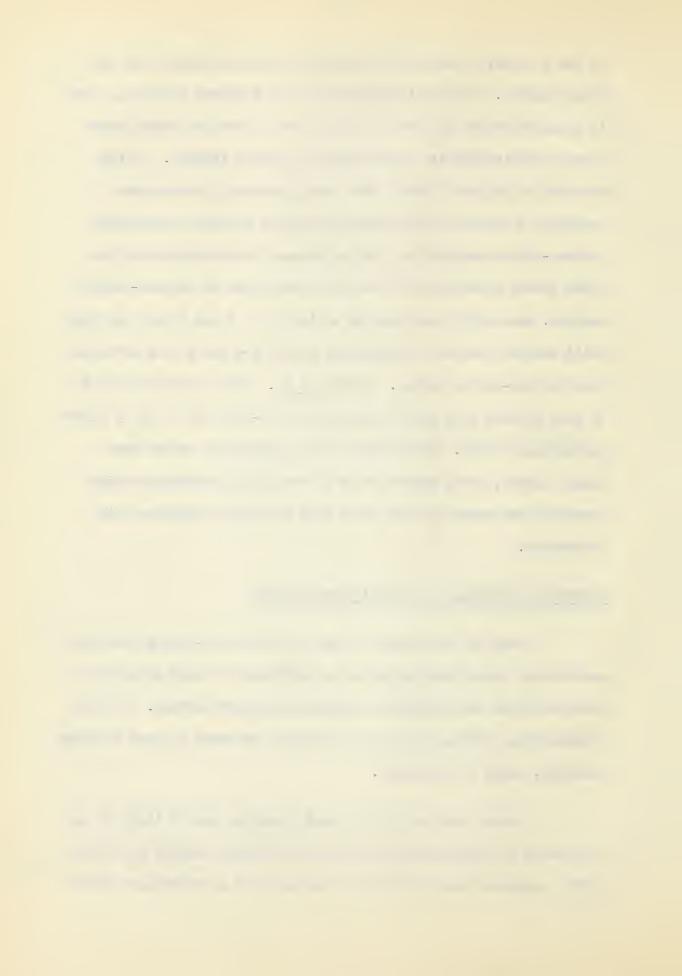


at the B-locus, a fact which suggested natural selection for the heterozygotes. Similar heterozygosity was observed at the A, B and to a lesser degree at the C, D and E loci of several other inbred lines of White Leghorns investigated by Briles (1956a). It was reported by Gilmour (1960a) that seven apparently independent antigenic loci were still segregating after fourteen consecutive brother-sister matings in a White Leghorn line studied while two other lines, after the 20th and 25th generation of brother-sister matings, were still heterozygous at the B, C, L and N loci and that still another line was heterozygous at the A-E and B loci after the 24th brother-sister mating. Briles et al. (1957) reported that two or more alleles were still present at the B-locus in 71 of 73 closed populations tested. Since most of the populations tested were highly inbred, there seemed to be a heterozygous advantage which prevented the genes at this locus from becoming homozygous with inbreeding.

Economic Significance of Blood Group Alleles

Many of the workers in the poultry blood-typing field have carried out their investigations to determine if blood group genes have an effect on economically important characteristics. If such relationships exist, selection of parents, at least in part on blood genotype, might be indicated.

After studying the A, B and D loci of twelve lines of the University of California production bred flocks, Schultz and Briles (1953) reported that selection on the basis of a performance index



seemed to favour heterozygotes over homozygotes at the A locus. The birds which were heterozygous at the A locus were not superior to the homozygotes for any one character, e.g., full sister family size, number of eggs layed, production index, November egg weight but were generally superior for all characters combined. They also found that one class of B heterozygote was favoured more when the A locus was heterozygous than when homozygous. The same relationship held with A in regard to heterozygosity of B. Thus, interaction between loci was indicated. Briles (1956b) reported on the data collected on three Texas inbred White Leghorn lines, 22, 23 and 24. These data had been reported in a number of abstracts, Briles et al. (1953), Briles (1954) and Briles and Krueger (1955). After three years Briles found that the increase in hatchability of heterozygotes over homozygotes was 18.0 per cent considering the female genotype alone and 19.4 per cent considering male genotype alone and that these differences within sex were highly significant by chi-square test. In order to determine if the hatchability differences were due to the genotype of the embryo, he reorganized the data according to expected-heterozygosity. There was a significant difference between the 0 and 50 per cent expectedheterozygous groups in favour of the latter. Livability of chicks from heterozygous dams was generally superior to livability of chicks from homozygous dams with the difference becoming greater with age of chick. The effect of the B-locus genotype on growth rates was different in the three inbred lines. In line 23, B²B⁶ males and females were consistently heavier at 9 weeks than their homozygous counterparts, thus indicating overdominance. In line 24, data pooled over three years



indicated that the B⁷B⁷ males were heavier at 9 weeks than the B²B⁷ or B²B² males while the B²B⁷ females were heavier at the same age than B²B² or B⁷B⁷ females. The author hypothesized that in line 24 an additive gene (or genes) was closely linked to the B-locus and that this gene was also exerting an overdominant effect. Thus the additive gene (or genes) might have obscured the overdominant B-locus effect in the faster growing males while the B-locus overdominance would have a greater chance to act in the slower growing females. Briles (1956) reported an association of the red blood cell antigens with 6 week weight of New Hampshires.

In adult birds Briles (1956b) found that the B-locus heterozygotes (B^2B^7) in lines 22 and 24 out-produced both homozygotes (B^2B^2 and B^7B^7) in per cent hen-day production by about 4 and 6 per cent for lines 22 and 24 respectively over the better homozygote. It should be noted that per cent production was seldom greater than 50 per cent per genotypic class. As far as livability was concerned the B^7B^7 birds seemed to show higher livability than the B^2B^7 birds in line 22 while in line 24 the B^2B^7 birds were more viable than the B^7B^7 birds.

Gilmour has also presented evidence derived from the Reaseheath lines of highly inbred White Leghorns to indicate that heterozygotes at various loci are superior (Gilmour 1954, 1958, 1959). However, he found that of the five loci studied, only L exhibited consistent heterosis in the reproductive characteristics influencing family size. This heterosis was observed in the effect of sire's genotype on fertility and of dam's genotype on egg production. Not even the L



locus, however, repeatedly exhibited superior livability in heterozygous progeny. This observation is contrary to results to be published by Briles and Allen (in press as reported by Gilmour 1960a). They indicated that the B heterozygous progeny were superior in viability from fertilization forward. Some indication of heterosis for egg production was also observed.



STUDIES AT THE UNIVERSITY OF ALBERTA

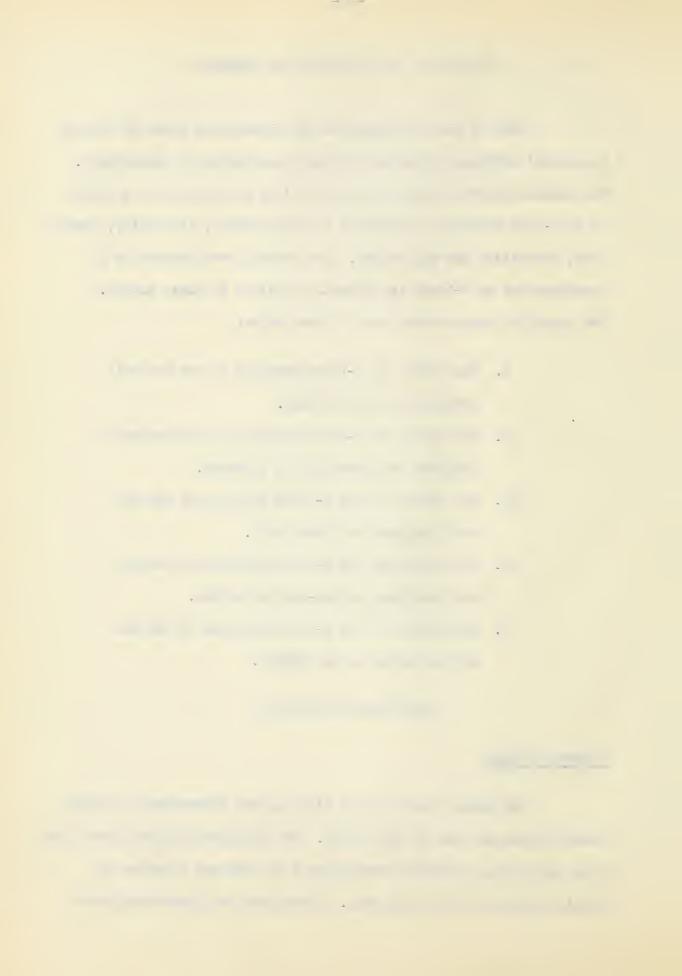
Most of the investigations on blood-group genes in poultry have dealt with egg production strains (see Review of Literature). The studies reported herein deal with blood group genes in a strain of meat-type chickens in relation to hatchability, livability, growth rate, production and egg weight. The studies were limited to a consideration of B-locus genotypes in relation to these traits. The specific areas studied are as shown below:

- I. The effect of B-locus genotype of the red cell antigens on hatchability.
- II. The effect of B-locus genotype of the red cell antigens on livability to 10 weeks.
- III. The effect of the B-locus genotype of the red cell antigens on growth rate.
 - IV. The effect of the B-locus genotype of the red cell antigens on hen-day production.
 - V. The effect of the B-locus genotype of the red cell antigens on egg weight.

Experimental (General)

Sources of Data

The White Plymouth Rock flock at the University of Alberta Poultry Farm was used in this study. The hatchability data were taken from the regular pedigree breeding pens of 1960 and a series of special matings in the same year. Livability and growth data were



collected on the progeny of the special matings of 1960. Additional growth data were obtained on stock hatched in 1961 from the regular pedigree matings. Egg production and egg size data were derived from production records of the progeny of the 1959 and 1960 regular pedigree matings.

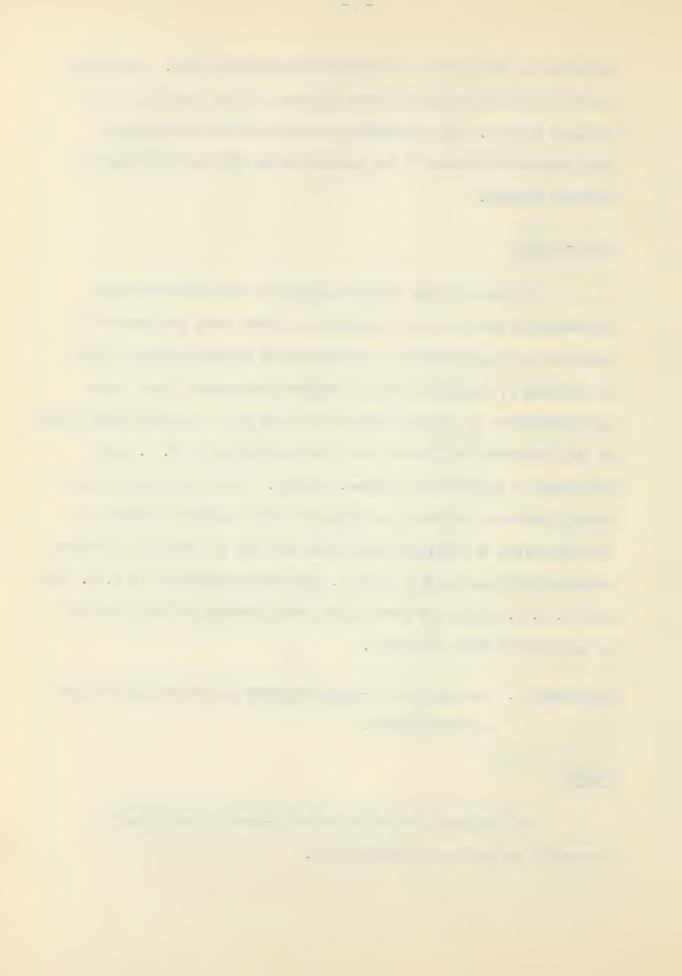
Blood-typing

A resume of the methods employed in the collection and processing of donor blood, immunization, titer test, preparation of antisera and classification of antisera into reagents will be found in Appendix A. Immunizations and related procedures, titer tests and preparation of antisera were done in the Animal Science Laboratories at the University of Alberta under the supervision of W. K. Barr, Department of Agriculture, Ottawa, Ontario. The work involved in the classification of antisera and typing of blood samples collected at the University of Alberta Poultry Farm was done at the Animal Diseases Research Institute at Hull, Quebec, under the supervision of W. K. Barr and Dr. S. S. Munro, the latter also being attached to the Department of Agriculture staff at Ottawa.

Experiment I. The Effect of B-locus Genotype of the Red Cell Antigens on Hatchability.

Object

To determine the effect of the B-locus heterozygosity (expected) of embryos on hatchability.

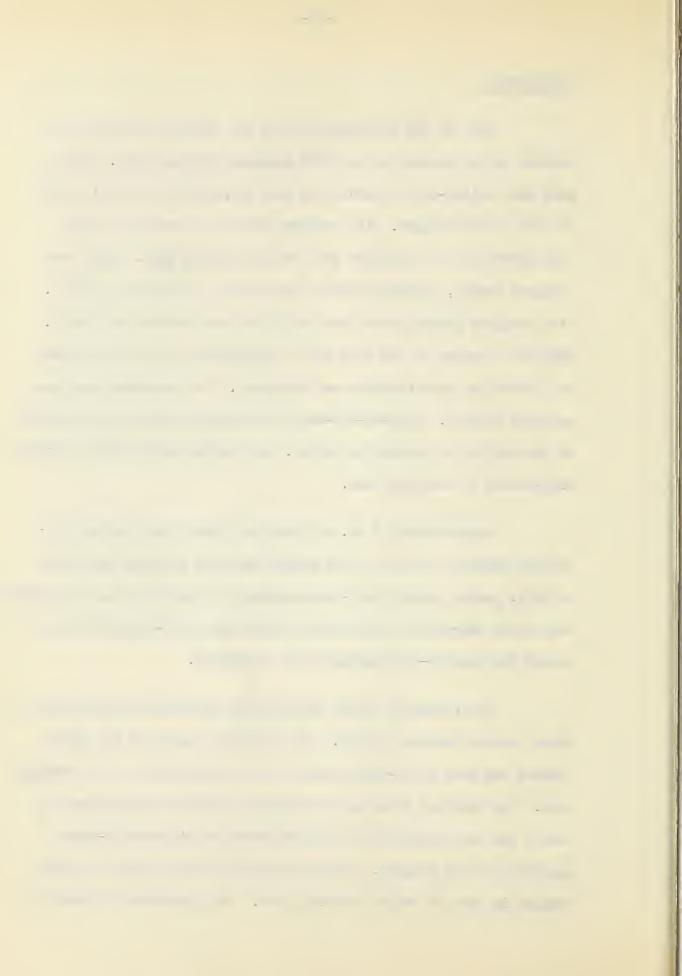


Experimental

Part of the hatchability data was obtained from the five regular spring hatches of the 1960 pedigree breeding pens. These pens were thirty-two in number and each consisted of one male mated to five or six females. All breeders were fed a breeding ration plus oyster shell, insoluble grit and water ad libitum. Eggs were pedigree marked, gathered several times daily and stored at 55° F. Five pedigree hatches were taken with one week between each hatch. Eggs were candled on the 18th day of incubation and all dead germs and infertiles were recorded and discarded. The remaining eggs were pedigree hatched. Dead-in-the-shell and chicks hatched were recorded at the end of the incubation period. All chicks hatched were pedigree wing-banded at hatching time.

Approximately 3 ml. of blood was taken from the wing vein of each breeder and sent to the Animal Diseases Research Institute at Hull, Quebec, where the B-locus genotype of the bird was established. Only about one-half of the breeding stock was blood-typed with the result that ninety-nine matings were considered.

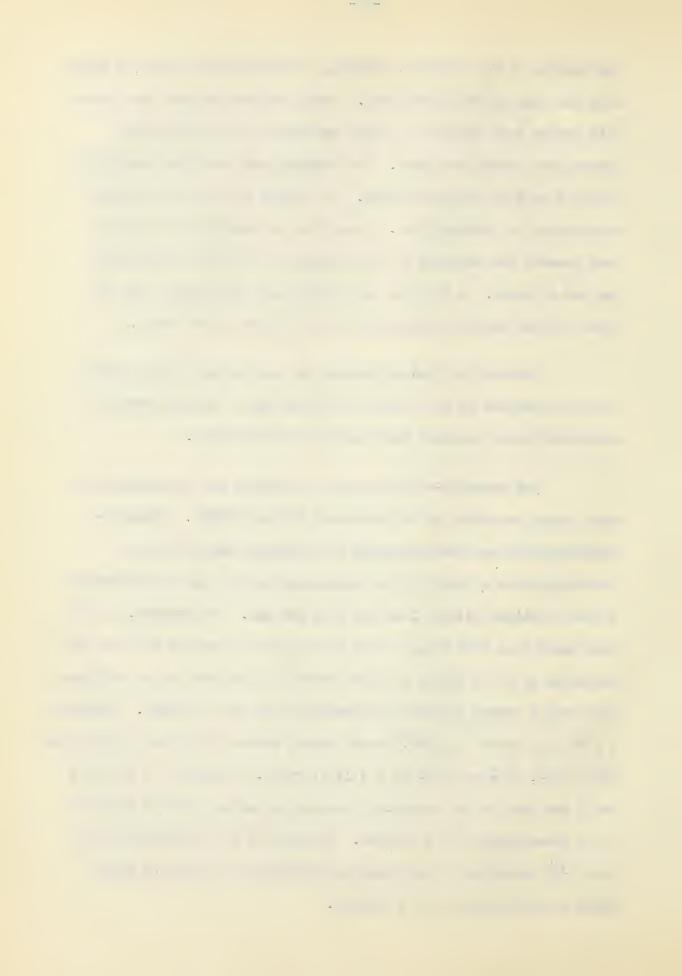
The remainder of the hatchability data was collected from three special hatches in 1960. All breeding stock used for these hatches had been blood-typed prior to the organization of the breeding pens. The breeding stock was distributed into the breeding pens in such a way as to produce the maximum number of different B-locus genotypes in the progeny. One male was placed with nine to sixteen females in each of twelve breeding pens. The procedures followed in



the feeding of the breeders, marking, collection and storage of eggs were the same as described above. Three pedigree hatches were taken with twelve days between the first and second and fourteen days between the second and third. The hatching and recording procedures followed were as described above. All chicks hatched were pedigree wing-banded at hatching time. Data from one breeding pen were not used because the accuracy of the genotype of the sire heading this pen was in doubt. A total of one hundred and two matings from the three special hatches were used in this portion of the study.

Hatchability was calculated for each mating as the number of chicks hatched as a per cent of fertile eggs. Matings were not considered which produced less than four fertile eggs.

each mating according to the method of Briles (1956b). Expected-heterozygosity was the percentage of offspring expected to be heterozygous as a result of the chance segregation and recombination of the B antigen alleles from the sire and dam. For example, a BlBl male mated to a BlB2 female would be expected to produce BlB1 and BlB2 offspring in a 1:1 ratio; in other words, 50 per cent of the offspring from such a mating should be heterozygous for the B alleles. Similarly a BlB2 male mated to a B2B3 female should produce offspring of genotypes BlB2, BlB3, B2B2 and B2B3 in a 1:1:1:1 ratio. Therefore, 3 out of 4 or 75 per cent of the offspring from such a mating would be expected to be heterozygous for B alleles. Similarly a BlB2 individual mated to a B3B4 individual should produce progeny 100 per cent of which would be heterozygous for B alleles.



In order to analyze the data, the hatchability of each mating was classified into 1 of 4 groups depending on whether the mating should produce 0%, 50%, 75% or 100% heterozygous offspring. These hatchabilities, which were expressed in per cent, were normalized by means of an arcsin transformation, Snedecor (1956), and the transformed data were analyzed by means of an analysis of variance, Goulden (1956).

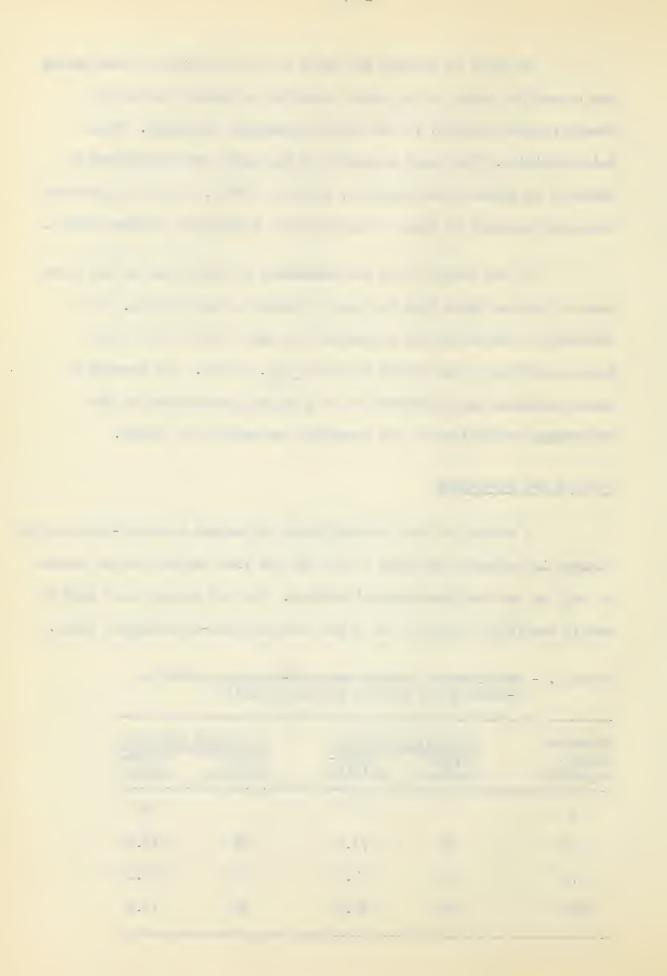
It was thought that the inbreeding of the chicks in the three special hatches might have had some influence on the results. The inbreeding coefficient was calculated for each chick of the first hatch according to the method of Rice et al. (1957). The average of these estimates was considered to be a valid approximation of the inbreeding coefficient of the remaining two hatches of chicks.

Results and Discussion

A summary of the hatchabilities of various expected-heterozygous classes is presented in Table 1 both for the five regular spring hatches as well as for the three special hatches. Too few matings were made to obtain meaningful data for the 0 per cent expected-heterozygous class.

Table 1. - Relationship between expected-heterozygosity for B-blood group alleles and hatchability

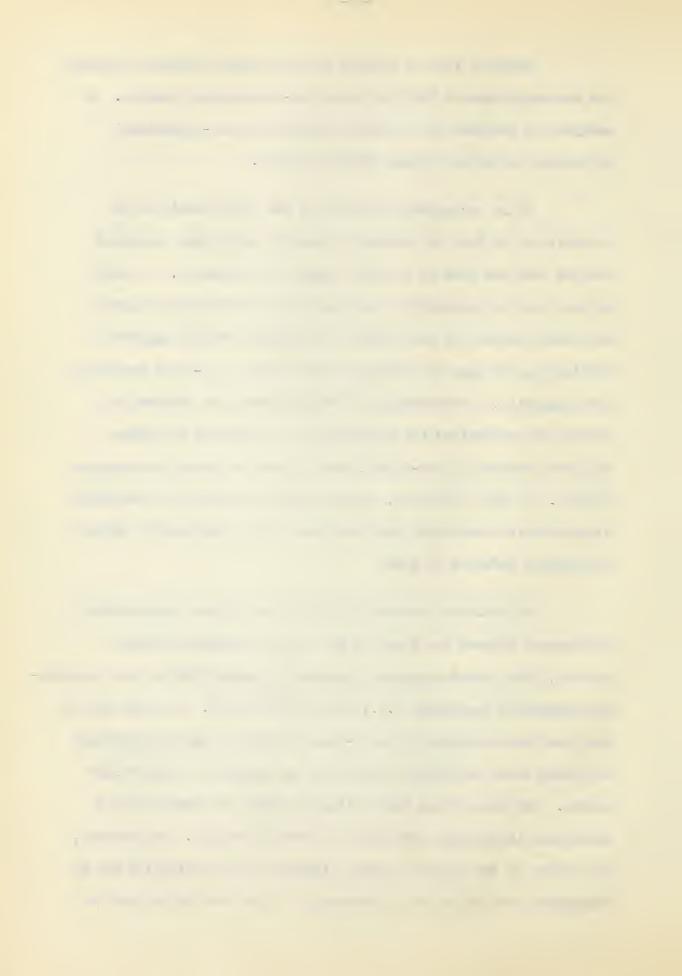
Expected Hetero- zygosity	5 Regular Number Matings	Hatches Hatch- ability	3 Special Number Matings	Hatches Hatch- ability
%		H		%
50	16	91.6	614	76.0
75	53	87.8	28	75.1
100	30	88.3	10	79.7



Study of Table 1 reveals no outstanding difference between the hatchabilities of different expected-heterozygous classes. An analysis of variance of the data resulted in a non-significant difference in hatchabilities between classes.

It is recognized that some of the eggs classified as infertile at 18 days of incubation actually could have contained embryos that had died at an early stage of development. In order to rule out the possibility that such errors might have affected the above results, it was decided to determine whether apparent fertility at 18 days of incubation was related to B-locus expected-heterozygosity. Substitution of fertility data (as observed at 18 days of incubation) for hatchability data used in the above analyses resulted in non-significant differences among heterozygous classes. It was, therefore, concluded that any errors in fertility classification that might have been made did not materially affect the results referred to above.

In addition to reporting highly significant hatchability differences between the 0 and 50 per cent heterozygous classes, Briles (1956b) noted a general increase in hatchability as the expected-heterozygosity increased, i.e., 50% to 75% to 100%. It could well be that the heterozygosity at the B-locus produces a greater advantage in flocks which are highly inbred such as those with which Briles worked. The three Texas inbred lines on which he reported had a calculated inbreeding coefficient of from 52% to 59%. In contrast, the chicks of the special hatches studied at this University had an inbreeding coefficient of approximately 7% and the chicks from the



regular hatches were even less inbred. Thus any hybrid vigour for hatchability that might have resulted from the heterozygosity at the B-locus may have been small compared to the positive or negative effects possible from many other genes.

It has been shown that other blood group loci, e.g.,

A-E, C, D, L and N also exist in addition to the B locus (see Review
of Literature). Although they were not considered in this analysis
these additional loci could have confounded the hatchability effects
of the B-locus.

Summary

(1) B-locus heterozygosity of the embryo does not seem to influence hatch of fertile eggs in this flock.

Experiment II. The Effect of B-locus Genotype of the Red Cell
Antigens on Livability to Ten Weeks.

Object

To determine the effect of B-locus heterozygosity on livability to 10 weeks of age.

Experimental

For part of Experiment II it was necessary to know the B-locus genotypes of the chicks from the three special hatches of 1960. Therefore, at approximately 11 weeks of age, about 2.5 ml. of blood was drawn from the left wing of each bird into a 3 ml. tube containing sodium citrate. Blood samples were packed in a box with

· . 1.* * . -, . _____ A A II

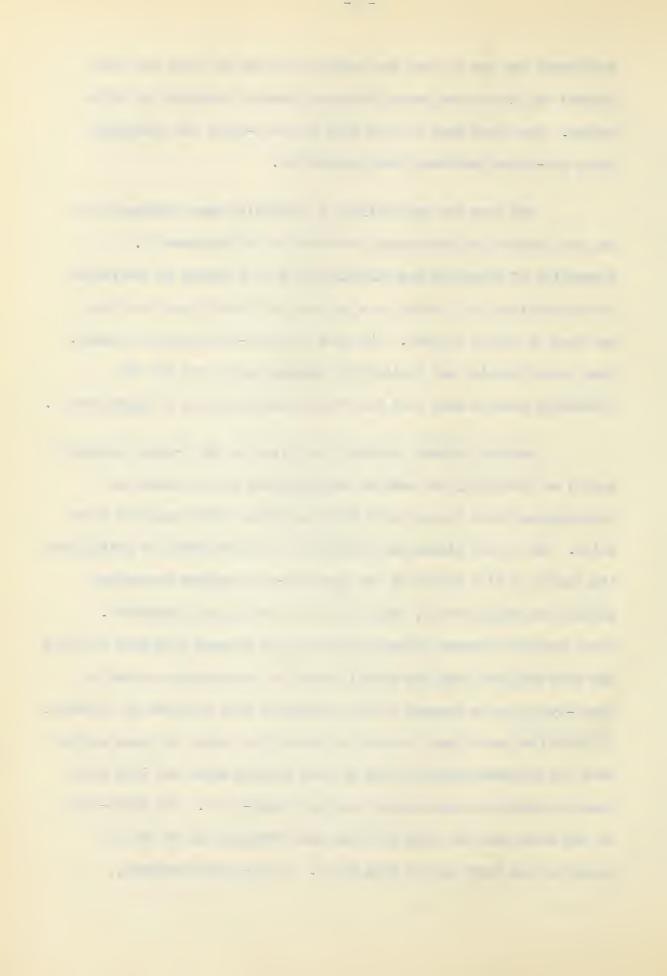
sufficient dry ice to keep the samples cool for 24 hours and were shipped via air to the Animal Diseases Research Institute at Hull, Quebec. The blood type of each bird at the B-locus was identified using iso-immune antisera (see Appendix A).

The data for calculation of livability were obtained from the one hundred and two matings referred to in Experiment I.

Livability of offspring was calculated for each mating as the number of chicks alive at 10 weeks as a per cent of fertile eggs and as a per cent of chicks hatched. The same expected-heterozygous classes, data transformation and statistical analysis were used for the livability data as were used for the hatchability data of Experiment I.

Another attempt to study the effect of the B-locus heterozygosity on livability was made by comparing the actual number of heterozygous birds living at 10 weeks with the number expected to be alive. The latter figure was calculated for each mating by multiplying the number of live chicks by the expected-heterozygous percentage figure (see Experiment I) under which the mating was classified.

These expected numbers of heterozygotes were grouped into sire families and were compared with the actual number of heterozygotes found by blood-typing to be present in the respective sire families at 10 weeks. It should be noted that in order to reduce the chance of bias, matings were not included which had one or more progeny which had lost wing bands or which for some reason were not blood-typed. The blood-types of two sires were in doubt and thus data from only 10 of the 12 breeding pens were used in this study. A chi-square analysis,



Goulden (1956), was used to test for real differences between the observed and expected number of heterozygotes.

Results and Discussion

A summary of the livability to 10 weeks based on fertile eggs and on chicks hatched is presented in Table 2. As already mentioned in Experiment I, insufficient numbers were present to report a 0% expected-heterozygous class. It will be noted that the best livability occurs in the 100% expected-heterozygous class for livability of fertile eggs and in the 75% expected-heterozygous class for livability based on chicks hatched. The superiority of the best livability classes over the poorest livability classes was 5.2% for the livability based on fertile eggs and 3.9% for the livability of chicks hatched. These differences were not found to be significant by analysis of variance.

Table 2. - Relationship between expected-heterozygosity for B-blood group alleles and livability to ten weeks of age based on fertile eggs and on chicks hatched

Expected		Iivabi	ility
Hetero- zygosity	Number Matings	Based on Fertile Eggs	Based on Chicks Hatched
Z		%	%
50	64	69.1	90.3
75	28	70.8	94.2
100	10	74.3	93.1



Briles (1956b) observed an improvement in livability to 9 weeks of age in three Texas inbred lines with increasing expected-heterozygosity with highly significant differences being obtained between the 0 and 50% expected-heterozygous classes. The improvements in livability noted in Table 2 with increasing heterozygosity were not as great as those noted by Briles but the over-all livability in the University of Alberta White Rocks was also much higher. Two probable reasons for the disagreement with Briles' results is that no 0% expected-heterozygous class was available for comparison and that the B-locus heterozygosity would likely improve viability much more in the highly inbred lines (52% to 59%) with which Briles worked than in the comparatively non-inbred chickens (7%) used in this experiment.

The observed and expected number of B-locus heterozygous progeny and the differences in their numbers are shown by sire in Table 3.

A chi-square analysis, Goulden (1956), indicated a significant difference between the expected and observed number of heterozygotes. Upon closer examination of the data it was noted that sire 8 produced no homozygotes in his progeny that lived to 10 weeks. Although such a situation is possible it is not probable. It was thought that some errors could have been made in typing or that unexplained linkage caused 100% heterozygotes at the B-locus in this sire's progeny. In any case the data were reanalyzed with the progeny from sire 8 omitted. The chi-square was then found to



be non-significant. In spite of this non-significance the number of observed heterozygotes exceeded the number of expected heterozygotes in 6 out of 9 of the remaining sire groups.

Table 3. - Observed and expected number of heterozygotes for B-blood group alleles at ten weeks of age

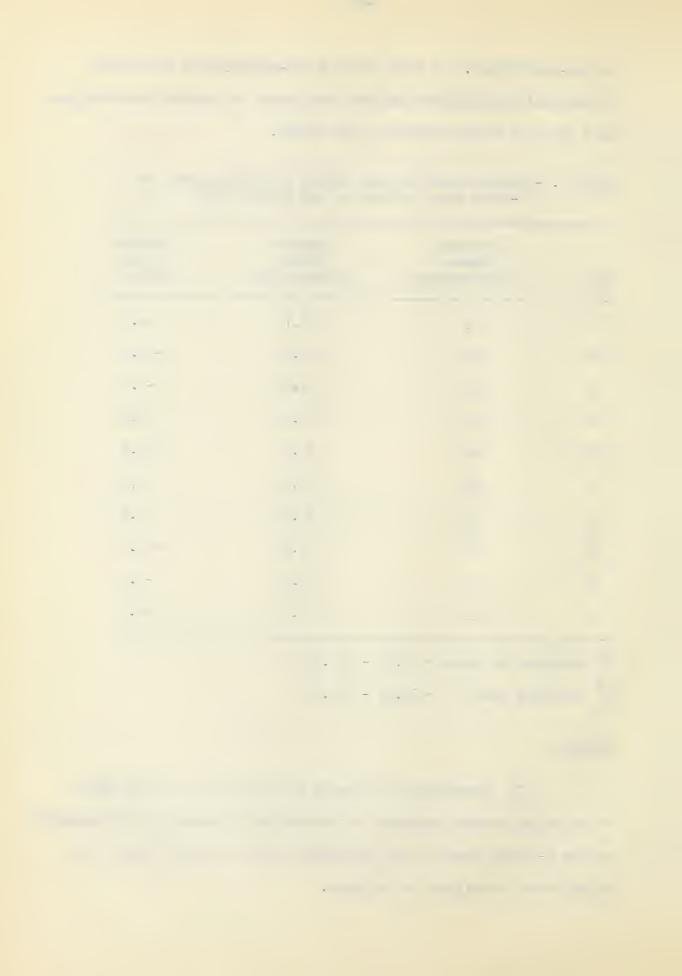
Sire	Observed Number Heterozygotes	Expected Number Heterozygotes	Observed Minus Expected
1	33	28.7	+4.3
2	η^{\dagger}	24.0	-10.0
3	15	19.0	-4.0
4	29	26.0	+3.0
5	40	30.0	+10.0
6	28	23.5	+4.5
7	35	34.5	+0.5
8	90	67.5	+22.5
9	31	35.0	-11.0
10	33	27.0	+6.0

 X^2 including 10 sires = 19.49 - P<.05

Summary

(1) Livability to 10 weeks of age based on fertile eggs or on chicks hatched appeared to increase with increasing heterozygosity at the B-locus, however, the differences noted were not found to be significant by analysis of variance.

 X^2 excluding sire 8 = 16.92 - P>.05



(2) Livability to 10 weeks of age seemed to be enhanced by B-locus heterozygosity as exemplified by the fact that more heterozygotes were alive at 10 weeks of age than might normally be expected as a result of the chance segregation and recombination of the B-alleles present. However, observed and expected numbers of heterozygotes did not differ significantly.

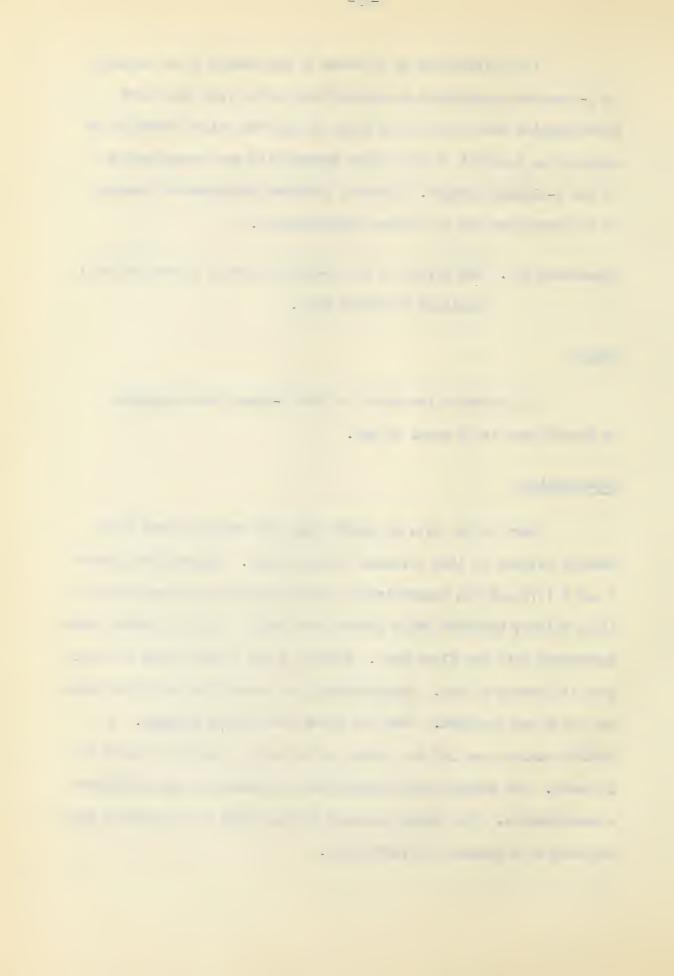
Experiment III. The Effect of the B-locus Genotype of the Red Cell
Antigens on Growth Rate.

Object

To determine the effect of the B-locus heterozygosity on growth rate to 10 weeks of age.

Experimental

Part of the data on growth rate was obtained from three special hatches of 1960 referred to previously. Chicks from hatches 1 and 2 (377 and 396, respectively) were randomized into raised wire floor battery brooders while chicks from hatch 3 (438 in number) were randomized into two floor pens. Hatches 1 and 2 were moved to floor pens at 2 weeks of age. Approximately one square foot of floor space per chick was provided. Feed and water were fed ad libitum. A broiler starter was fed to 6 weeks and a broiler finisher from 6 to 10 weeks. The starter and finisher were in mash form and contained a coccidiostat. The weight in grams of each bird at 10 weeks of age was used as a measure of growth rate.



Some of the chick growth data which was obtained from these three special hatches had to be discarded. The genotypes of two of the sires used in the breeding pens were uncertain and therefore their progeny were omitted from the analysis. In addition, certain chicks whose genotypes were ambiguous or which were not typed could not be used in the analysis. Birds that were retarded in growth as a result of perosis also were not used. A total of 799 chicks were considered in the analysis.

The data were difficult to analyze for purposes of making inferences. Because of the unequal frequency of alleles in the parental population it was impossible to have each genotype represented by the same number of birds. It is also obvious that each sire could not produce every B-locus genotype. The data were therefore non-orthogonal and were treated according to the method described under Appendix B.

Since there was a possibility that inbreeding might influence the results, a correlation analysis between inbreeding coefficient and 10-week weight was conducted on the birds of the first hatch as a measure of this possible effect.

The other part of the data on growth rate was collected on the 2nd, 3rd and 4th hatches of the five regular spring hatches of the 1961 pedigree breeding pens. These hatches were produced in the same way as the regular pedigree hatches of 1960 which were described under Experiment I. The sires and dams were blood-typed in the manner already outlined. Progeny were grouped into 50%, 75%



or 100% expected-heterozygous classes as in Experiment I. The number of male and female chicks involved in each expected-heterozygous class is shown in Table 7. An analysis of variance, Goulden (1956), was conducted on the 6-week chick weights of the chicks so grouped.

Results and Discussion

A summary of the correlation analysis is presented in Table 4. The correlation coefficients of -.058 for males and -.012

Table 4. - Correlation between ten-week body weight and inbreeding coefficient of the chicks

Sex	Number	$\mathbf{r}_{\mathbf{x}\mathbf{y}}$
Males	124	 058 [#]
Females	163	012#

[#] Correlation coefficient not significant at .05 level of probability.

for females were negative but were not significant at the 5% level of probability, Snedecor (1956). Since the chicks from the 1961 breeding pens were even less inbred than the chicks from the special pens, it was concluded that the inbreeding of the chicks had no effect on the growth results to 10 weeks of age.

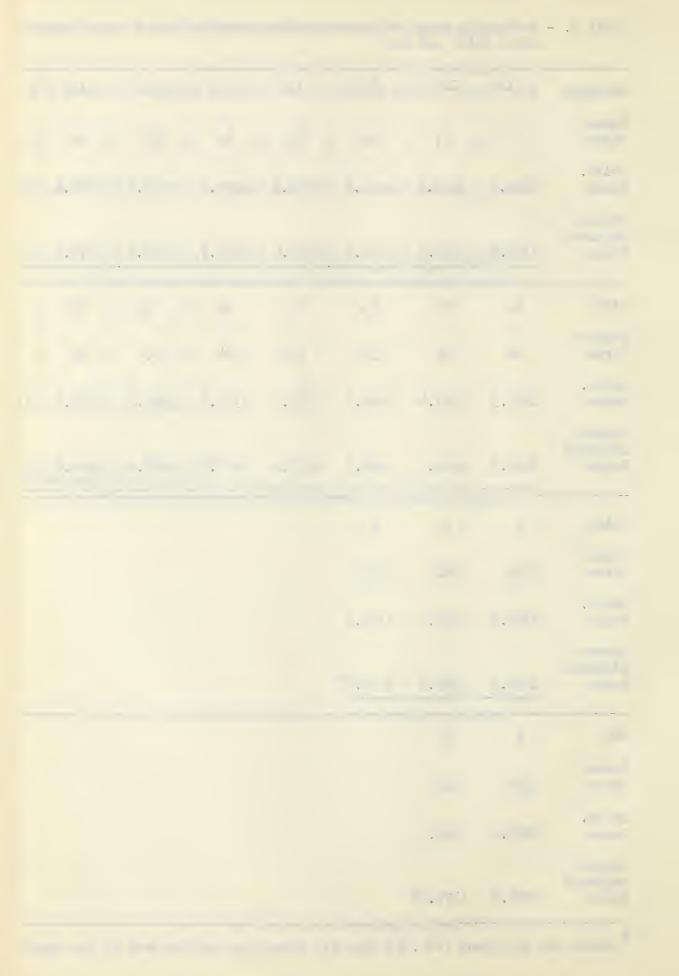
A summary of the arithmetic means and the ranked adjusted means of 10-week chick weights is presented in Table 5. The first section of Table 5 shows the effect of genotype on 10-week chick weights adjusted for sire, hatch and sex. Although the adjusted means ranged from a low of 1366.9 to a high of 1547.5 grams, an



Table 5. - Arithmetic means and corresponding ranked adjusted means (grams) of ten-week body weight classified according to genotype, sire, hatch and sex

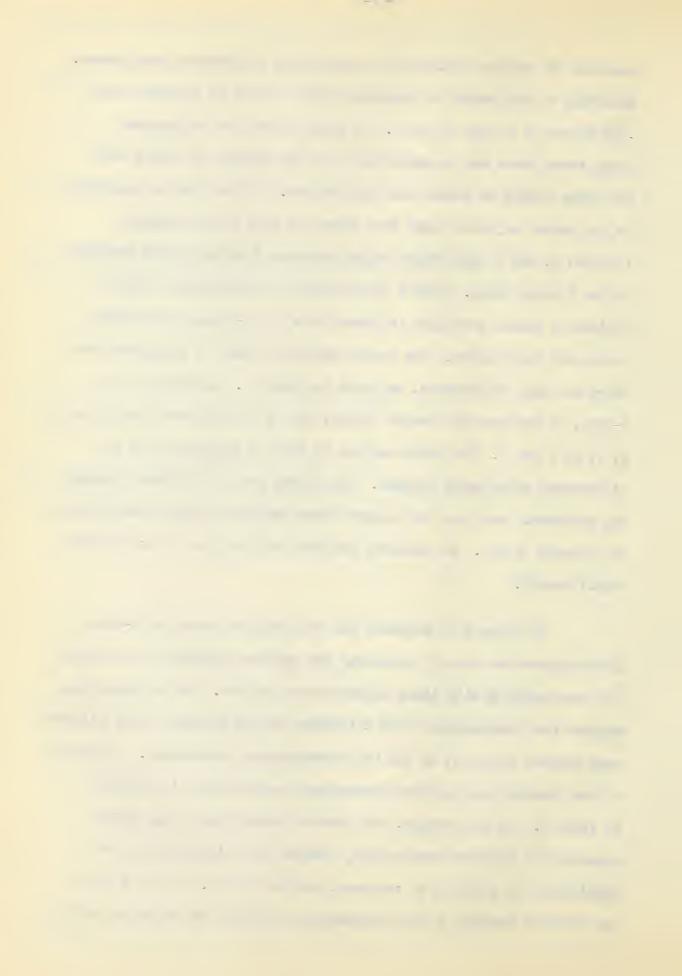
	D220, 11.	, and s	COS.											
Genotype	B1B12	B12B12	BrtBrt	B _J B _l t	B2 B/t	B1B2	BlBl	B3B3	B1B3	_B 2 _B 3	B ² B ²	_B 4 _B 12	B3B4	B3B12
Number Birds	43	17	46	25	49	55	72	137	94	81	34	43	78	25
Arith. Means	1404.3	1401.2	1264.8	1290.2	12%.6	1360.3	1378.2	1384.8	1378.3	1377.0	1322.6	1372.4	1381.2	1433.6
Ranked Adjusted Means	1366.9	ग्रोगे6•2	1451.2	1462.4	1474.7	1474.8	1477.8	1478.5	1479.8	1484.5	1497.3	1511.0	1519.9	1547.5
Sire	s ₆	s ₂	s ₃	s ₇	s ₈	S ₁₀	S5	s ₉	s ₁	Sl				
Number Birds	814	614	54	86	104	63	78	91	94	81				
Arith. Means	1283.3	1341.4	1290.5	1353.2	1393.6	1347.8	1374.2	13%.4	1399.2	1422.9				
Ranked Adjusted Means	1416.3	1461.8	1464.7	1472.9	1478.5	1485.4	1486.0	1519.8	1520.8	1603.8#				
Hatch	3	1	2											
Number Birds	294	252	253											
Arith. Means	1374.9	1344.1	1374.2											
Ranked Adjusted Means	1478.5	1485.4	1491 .7 #											
Sex	F	М												
Number Birds	3%	403												
Arith. Means	1228.0	1499.5												
Ranked Adjusted Means	1207.5	1478.5#	,											

[#] Means are different (P<.05) from all others not underscored by the same line.



analysis of variance indicated no statistical differences among means. According to the method of Henderson (1960), there is a greater than .466 chance of a type II error. In other words, the differences among these means may be significant but the numbers of chicks were not large enough to detect such differences. It can also be observed in the ranked adjusted means that there are only 4 heterozygotes included in the 7 lower means while there are 5 heterozygotes included in the 7 upper means. Such a distribution of heterozygotes might indicate a slight advantage in growth rate for B-locus heterozygous birds over homozygotes. The second section of Table 5 indicates that there are sire differences, as might be expected. According to a t-test, as outlined by Fredeen (1961), sire 4 is different from sires 10, 8, 7, 3, 2 and 6. The third section of Table 5 indicates that no differences exist among hatches. The fourth section of Table 5 shows the arithmetic mean and the adjusted mean weights for males and females at 10 weeks of age. As expected the mean body weights by sex differed significantly.

An attempt to evaluate the overdominant effect of B-locus heterozygotes was made by comparing the observed adjusted mean weights for heterozygotes with their expected mean weights. The expected mean weights for heterozygotes were calculated as the average of the adjusted mean weights (Table 5) of the two corresponding homozygotes. A summary of the observed and expected heterozygote mean weights is presented in Table 6. On the average, the observed heterozygote mean weight exceeded the expected mean weight, however, the difference was not significant by analysis of variance, Goulden (1956). The fact that the observed weights of the heterozygotes exceeded the expected weights



in 5 out of 9 of the comparisons suggests that the heterozygotes were better than their corresponding homozygotes.

Table 6. - Observed adjusted means and expected means (grams) of body weight at ten weeks of age in heterozygotes

Genotype	Observed Mean Weight	Expected Mean Weight	Observed Minus Expected
B1B12	1366.9	1462.2	-95.3
B_1B_1	1462.4	1464.5	-2.1
B2B4	1474.7	1474.2	+ •5
$B^{1}B^{2}$	1474.8	1487.6	-12.8
$B^{1}B^{3}$	1479.8	1478.2	+1.6
B^2B^3	ग्रिशि•2	1487.9	-3.4
_B 4 _B 12	1511.0	<u> भि</u> भि8•8	+62.2
B3Bl4	1519.9	1464.8	+55.1
_B 3 _B 12	1547.5	1462.5	+85.0
Mean	1480.2	1470.1	+10.1

Table 7 shows a summary of the average weights at 6 weeks of the progeny of three 1%1 spring hatches. Analysis of variance for the data summarized in Table 7 is shown in Table 8. The term genotype in Table 8 refers to the expected-heterozygous class into which each chick falls, i.e., 50%, 75% or 100%. There was no significant difference among genotypes nor was there a significant sex times genotype interaction. There was, as expected, a highly significant difference between male and female weights. There was

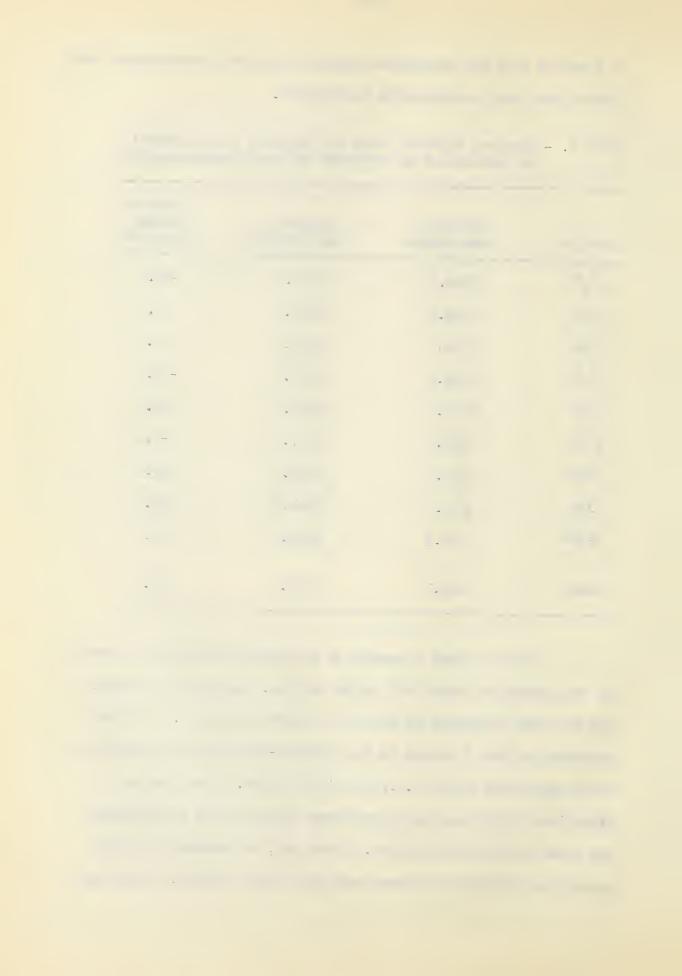


Table 7. - Relationship between expected-heterozygosity and body weight at six weeks of age

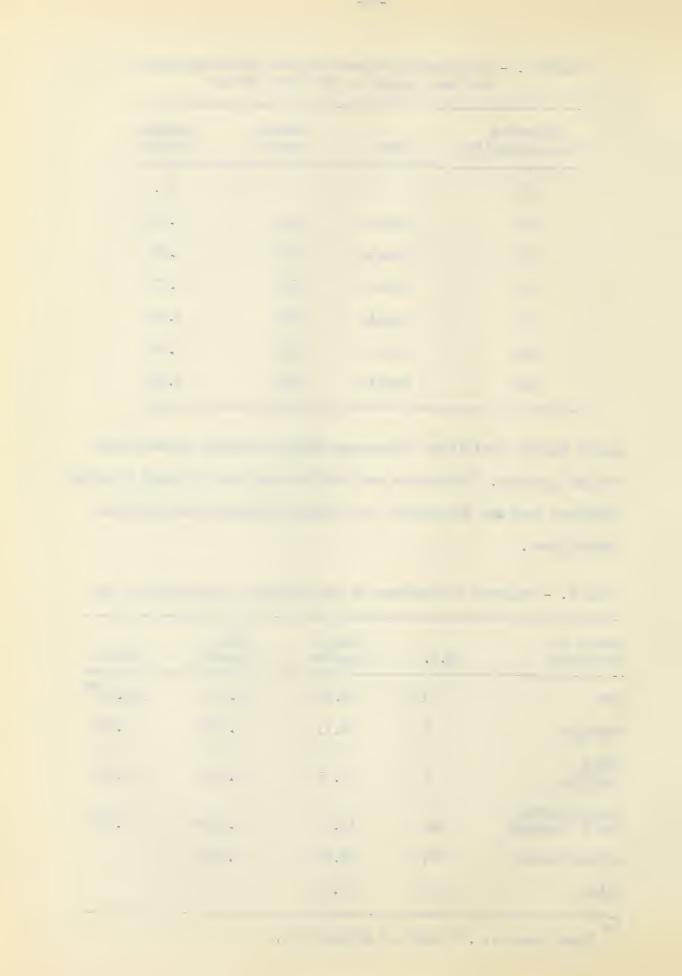
Expected Heterozygosity	Sex	Number Birds	Average Weight
8			1b.
50	Male	133	1.77
50	Female	130	1.50
75	Male	188	1.77
75	Female	266	1.50
100	Male	202	1.80
100	Female	21վե	1.50

also a highly significant difference between families taken within sex and genotype. These were dam families and thus it would seem that different dams had the ability to produce offspring with different growth rates.

Table 8. - Analysis of variance of body weight at six weeks of age

Source of Variation	D.F.	Sums of Squares	Mean Square	F Ratio
Sex	1	22.39	22.39	374.41**
Genotype	2	0.13	0.065	1.08
Sex X Genotype	2	0.01	0.005	0.08
Family within Sex & Genotype	260	15.56	0.0598	1.83**
Within Family	897	29.27	0.0326	
Total	1162	67.36		

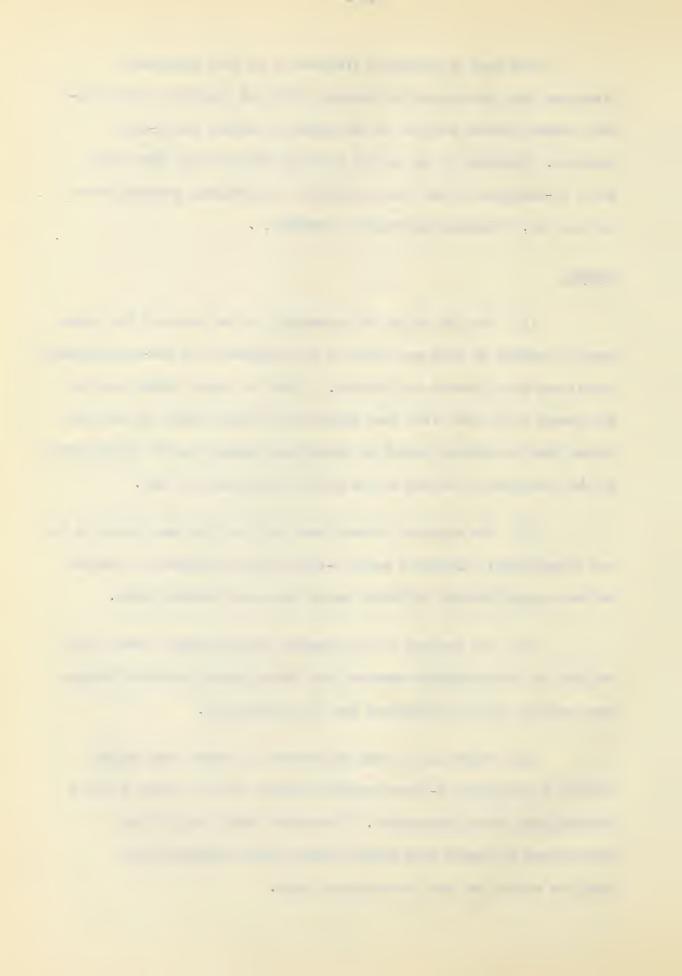
^{**} Significant at .01 level of probability.



The lack of genotypic differences in this experiment disagrees with the results of Briles (1956) who reported a relation-ship between 6-week weights in New Hampshire chicks and B-locus genotype. Possibly if the chicks of these 1961 hatches were typed and a re-analysis of the data according to individual genotype were carried out, relationships might be evident.

Summary

- (1) The low degree of inbreeding in the stock of the three special hatches of 1960 was found to be negatively but non-significantly correlated with 10-week body weight. Since the other chicks used in the growth study were even less inbred than these chicks, it was concluded that the slight amount of inbreeding present in the chicks used in the study had no effect on the growth to 10 weeks of age.
- (2) The adjusted 10-week mean body weights were found to be not significantly different among B-locus blood genotypes or hatches but were significantly different among sires and between sexes.
- (3) The average of the observed adjusted mean 10-week body weights for heterozygotes exceeded the corresponding expected average mean weights but the difference was not significant.
- (4) There was no real difference in 6-week body weight between heterozygous B-locus genotype classes nor was there a sex by heterozygous class interaction. There were highly significant differences in 6-week body weight between sexes and among dam families within sex and heterozygous class.



Experiment IV. The Effect of the B-locus Genotype of the Red Cell
Antigens on Hen-Day Production.

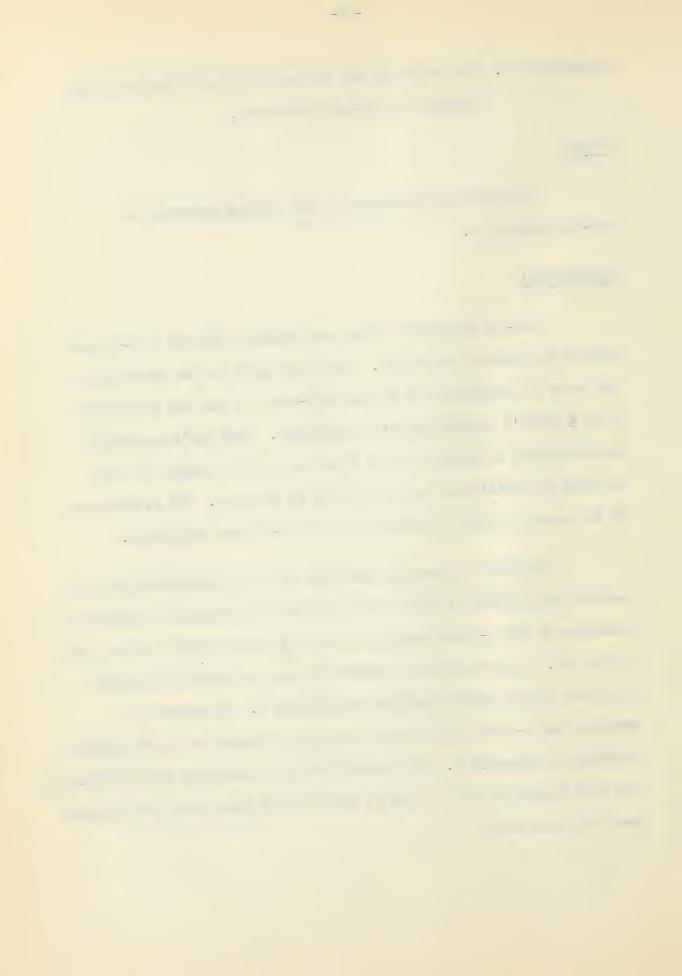
Object

To determine the effect of the B-locus genotype on hen-day production.

Experimental

Hen-day production data were obtained from the 1959-60 and 1960-61 egg production records. Only eggs layed in the period from the start of consistent lay in the trap-nests to the end of December (4 to 5 month's production) were considered. Each hen's production was calculated as number of eggs layed of the total number of days on which she could have layed expressed as per cent. The productions of 214 hens in 1959-60 and 451 hens in 1960-61 were considered.

In order to normalize the data an arcsin transformation was carried out, Snedecor (1956), and the information was then classified according to the B-locus genotype of the hen and according to the sire of the hen. A matrix of the transformed data was formed and solved according to the method described in Appendix B. An analysis of variance and t-tests were conducted where applicable and in the manner described in Appendix B. The average hen-day percentages were essentially the same regardless of the date of hatch of the layers and thus hatches were not considered.



Results and Discussion

A summary of the 1959-60 hen-day productions is presented in Table 9. The unadjusted arithmetic means for genotypes range from 64.7% for BlBl to 72.6% for BBl2, a difference of 7.9. The adjusted means for genotype range from a low of 66.5% to a high of 77.5%, a difference of 11. An analysis of variance indicated that there were no significant differences among adjusted means. A similar analysis of the 1960-61 production records indicated no significant differences among genotypes for hen-day production.

These results disagree with those of Briles (see Review of Literature) who found that the B-locus heterozygotes in two inbred lines (22 and 24) out-produced the better homozygotes in hen-day production by approximately 4 and 6 per cent respectively. Although the hen-day production advantages for heterozygotes were consistent over the 3 years, Briles' population numbers were small becoming as low as 6 pullets housed for line 22 in 1951. The 1959-60 and 1960-61 egg production records discussed in this experiment indicated no consistent advantages for B-locus heterozygotes over homozygotes or vice versa. One possible reason for the disagreement between these results and those of Briles is that the lines with which Briles worked were more highly inbred than the University of Alberta White Rocks. The B-locus heterozygotes might show more consistent effects in Briles' flocks than in the relatively non-inbred birds studied in this experiment.

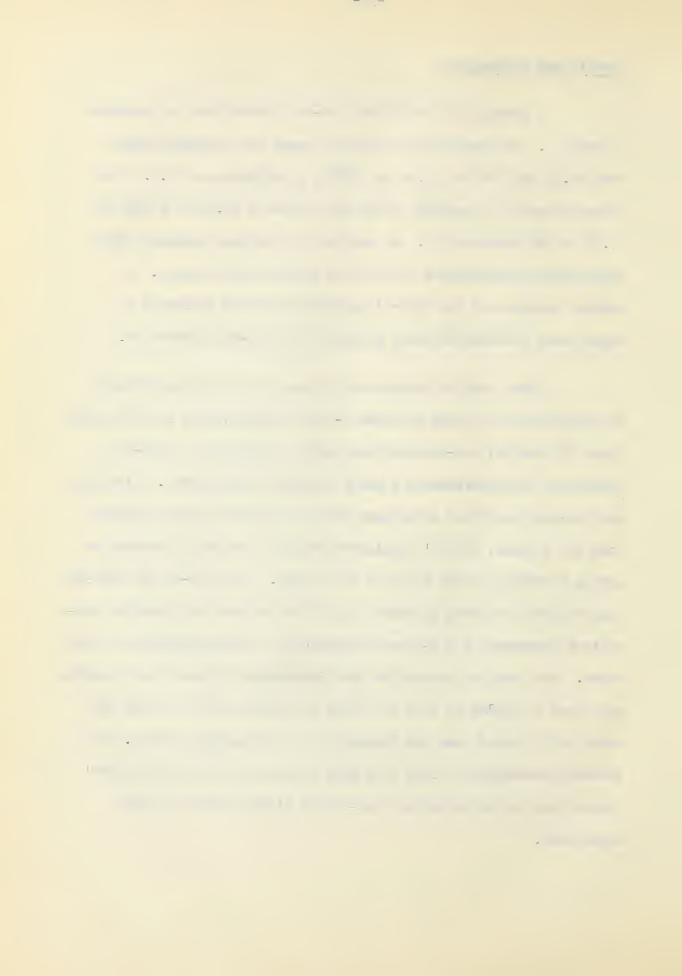
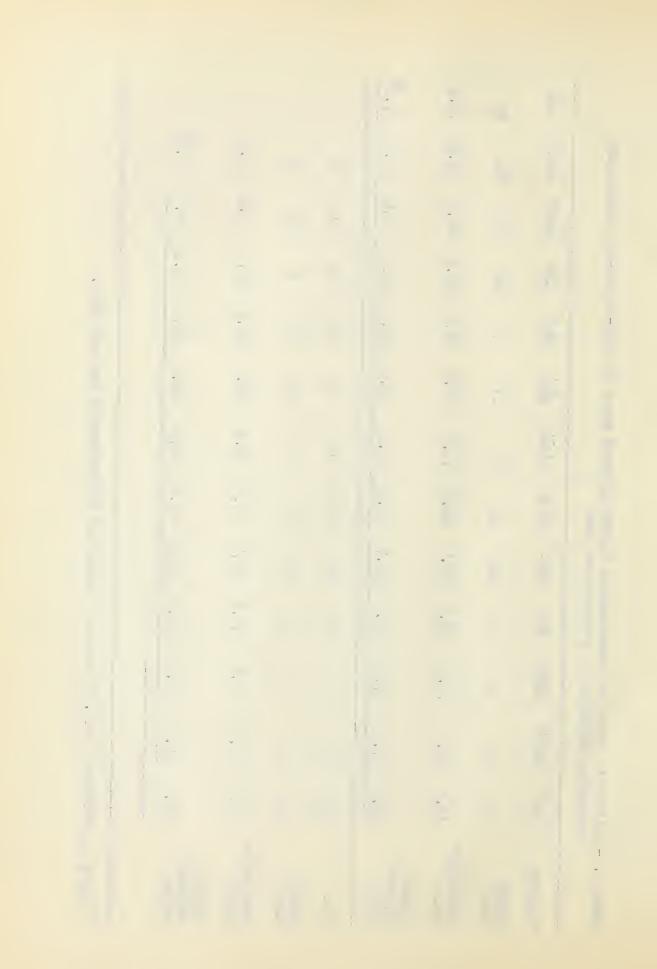


Table 9. - Arithmetic means and corresponding ranked adjusted means of 1959-60 hen-day productions classified according to genotype and sire

Genotype	$_{\rm B}$ $_{\rm B}$	B ^d BJ2	B3B12	Bub	B ² B ³	$^{\mathrm{B}1\mathrm{B}2}$	$_{\rm Bl_{Bl}^{22}}$	B ² B ⁴	$_{\rm B^{1}B^{1}}$	B3B4	B ¹ B ⁴	$_{\rm B}$ $_{\rm B}$ $_{\rm B}$	B ³ B ³
Number Birds	9	7	7⁄	2	16	84	22	21	2	7,5	19	21	97
Arithmetic Means	67.5	9.59	72.6	ħ•89	4-69	9.89	71.8	1.69	64.7	66.4	69.5	68.5	71.4
Ranked Adjusted Means	66.5	0.79	67.8	68.6	1.69	68.6	71.3	71.5	71.8	72.6	76.4	76.8	17.5#
Sire	S ₁ 5	6S	85	S11	TS'	818	\$20	S ₃	न्तर	S ₁₂	S ₁₉	s _s	
Number Birds	16	17	ω	帮	20	큐	21	76	Ħ	77	36	17	
Arithmetic Means	63.8	ħ•19	4.69	71.0	4.79	9.19	69.1	8.89	4-69	69.1	4.69	76.5	
Ranked Adjusted Means	56.4	62.1	63.7	8.46	8.99	9.29	68.3	8.89	68.9	69.1	8.69	76.6#	

Means are different (P<.05) from all others not underscored by the same line.



It is an accepted fact that sires can influence the performance of their progeny to a considerable degree. The lower section of Table 9 shows that the adjusted mean hen-day productions range from a low of 56.4% for sire 15 to a high of 76.6% for sire 8, a difference of 20.2. Significant differences (t-test) were found among sires as indicated in the table. Sire differences were not tested in 1960-61.

Summary

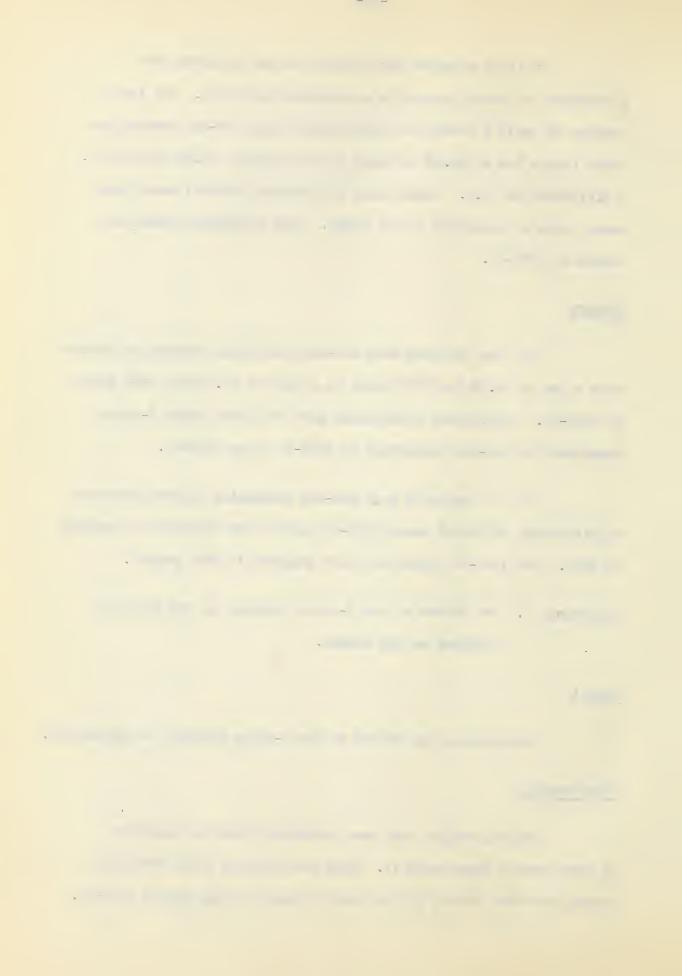
- (1) The adjusted mean hen-day production percentages varied from a low of 66.5% for B¹B⁵ birds to a high of 77.5% for B³B³ birds in 1959-60. Significant differences were not found among B-locus genotypes for hen-day production in 1959-60 or in 1960-61.
- (2) The adjusted mean hen-day production percentages were significantly different among 1959-60 layers when classified according to sire. The 1960-61 layers were not analyzed in this regard.
- Experiment V. The Effect of the B-locus Genotype of the Red Cell
 Antigens on Egg Weight.

Object

To determine the effect of the B-locus genotype on egg weight.

Experimental

The egg weight data were collected from the same hens as were used in Experiment IV. Eggs were weighed twice weekly in ounces per dozen during 1959-60 and in grams per egg during 1960-61.



Average egg weight for each hen was calculated from the weights of all normal eggs layed during the first 3 months of lay. The average egg weights so obtained were then grouped according to B-locus genotype and sire of the layer. Statistical treatment was similar to that used in Experiment IV and is described under Appendix B.

Results and Discussion

A summary of the average egg weights for 1959 is presented in Table 10. Although the adjusted means vary from 22.1 to 24.7 ounces per dozen there was no significant difference among the means for B-locus genotypes. It can be observed that the only two homozygous classes of the series, B³B³ and B¹B¹ have the heaviest adjusted mean weights. The lower section of Table 10 shows the adjusted mean egg weights for layers classified according to sire. A t-test indicated significant egg weight differences among sires.

The data from the 1960 production records present a somewhat different picture in Table 11. The adjusted mean egg weights for various B-locus genotypes varied from a low of 49.1 to a high of 54.4 grams per egg. An analysis of variance indicated a highly significant difference among genotypes for 3 month average egg weight. The fact that the eggs layed during the first year of the study were weighed on a spring-type scale in ounces per dozen, whereas, the eggs layed in the second year of the study were weighed on a very accurate shadow graph type scale in grams per egg may have been responsible for the variability in results obtained in the two years.

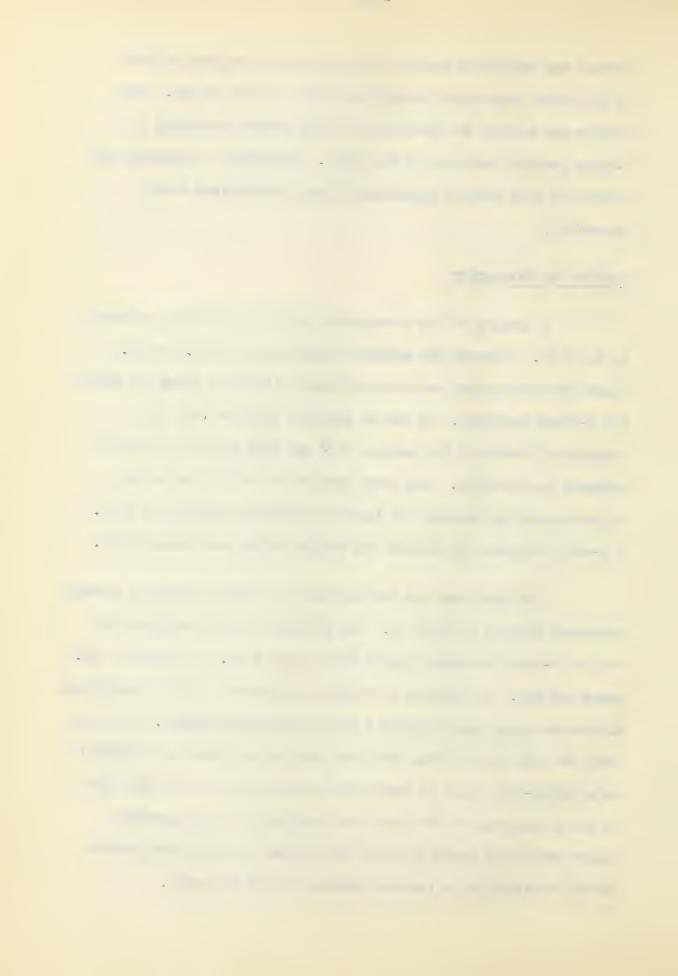


Table 10. - Arithmetic means and corresponding ranked adjusted 1959 average egg weights (oz./doz.) classified according to genotype and sire

Genotype	B ² B ⁴	B3B12	B4B12	B4B5	BlB5	B ² B ³	B1B12	BB	ВЗВф	BlB3	BIB	B.B.	BlBl
Number Birds	21	N	11	7	9	16	22	81	15	21	19	97	2
Arithmetic Means	23.0	22.8	23.1	22.6	23.3	23.1	24.0	24.0	21.9	22.3	22.5	22.7	23.1
Ranked Adjus ted Means	22.1	22°T	22.6	22.6	23.2	23.2	23.h	23.5	23.7	23.8	23.9	24.42	24.7#
Sire	8. 5.	S11	818	So	315	S ₁₉	S ₁₂	S.	s S	აე ¬	T _S	\$20	
Number	æ	34	큐	17	16	36	7	97	17	20	7	21	
Arithmetic Means	21.5	22.2	21.5	22.8	22.6	23.0	23.5	24.3	24.0	23.8	24.0	1-42	
Ranked Adjusted Means	21.2	21.6	21.7	22.1	22,2	23.5	23.5	24.4	24.5	24.5	24.5	24.9#	

Means are different (P<.05) from all others not underscored by the same line.

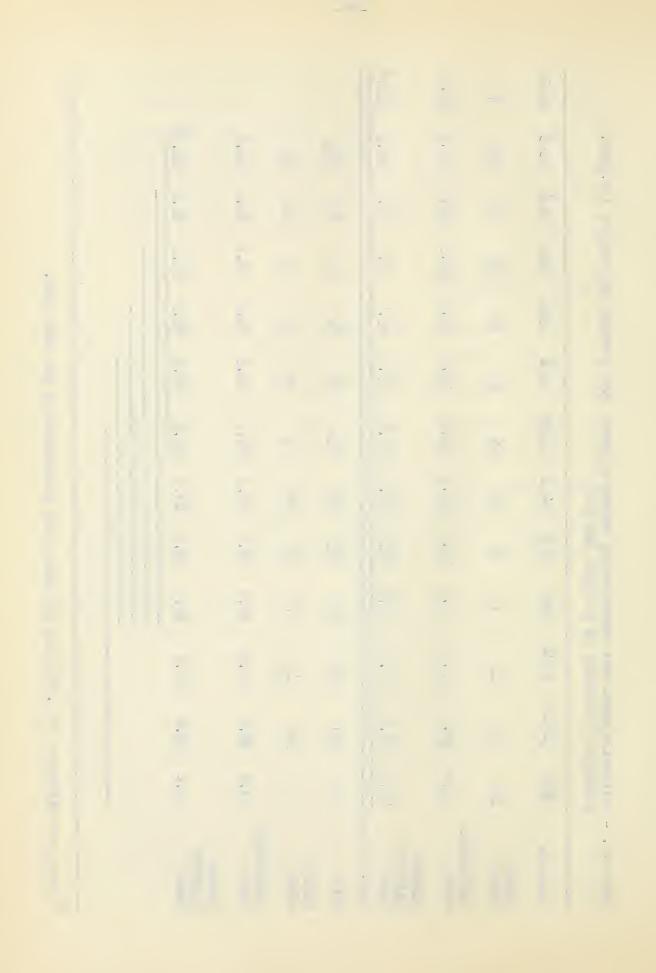


Table 11. - Arithmetic means and corresponding ranked adjusted 1960 average egg weights (grams) classified according to genotype

Genotype	B12B12	Bh B12	_B 3 _B 12	B1B12	BrtBrt	_B 2 _B 12	B1B5	B3B4	B1B1	BIBH	_B ² _B 3	в ² в ²	_B 3 _B 3	_B 1 _B 2	B ₂ B∤t	B ₁ B ₃
Number Birds	5	13	114	314	6	15	5	26	35	54	39	<u> 1</u> 1+	25	61	22	83
Arithmetic Means	49.2	51.8	52.6	52.4	52.3	52.8	54.8	52.6	53.2	53.7	54.0	53.9	52.4	54.5	55.0	53.7
Ranked Adjusted Means	49.1	50.4	51.7	52.0	52.8	52.8	52.9	52.9	53.3	53.5	53.6	53.8	53.8	54.3	54.4	54.4#

Means are different (P<.05) from all others not underscored by the same line.

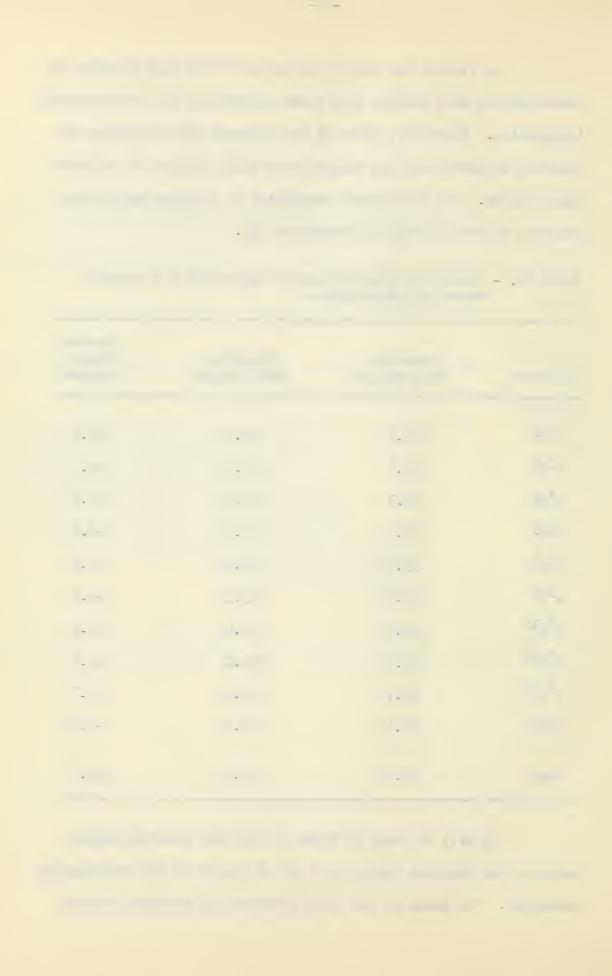


An attempt was made to determine if the eggs produced by heterozygotes were heavier than those produced by the corresponding homozygotes. Therefore, Table 12 was prepared which contains the observed adjusted mean egg weights from Table 11 plus the expected mean weights. The latter were calculated in the same way as the expected values in Table 6, Experiment III.

Table 12. - Observed adjusted means of egg weight and expected means in heterozygotes

Genotype	Observed Mean Weight	Expected Mean Weight	Observed Minus Expected
B_B^{1}	54.4	53.6	+0.8
B2Blt	54.4	53.3	+1.1
B1B2	54.3	53.6	+0.7
B^2B^3	53.6	53.8	-0.2
BlBh	53.5	53.0	+0.5
B3Blt	52.9	53.3	-0.4
_B 2 _B 12	52.8	51.4	+1.4
B1B12	52.0	51.2	+0.8
$_{\rm B}$ 3 $_{\rm B}$ 12	51.7	51.4	+0.3
_B 4 _B 12	50.4	51.0	-0.6
Mean	53.0	52.6	+0•11

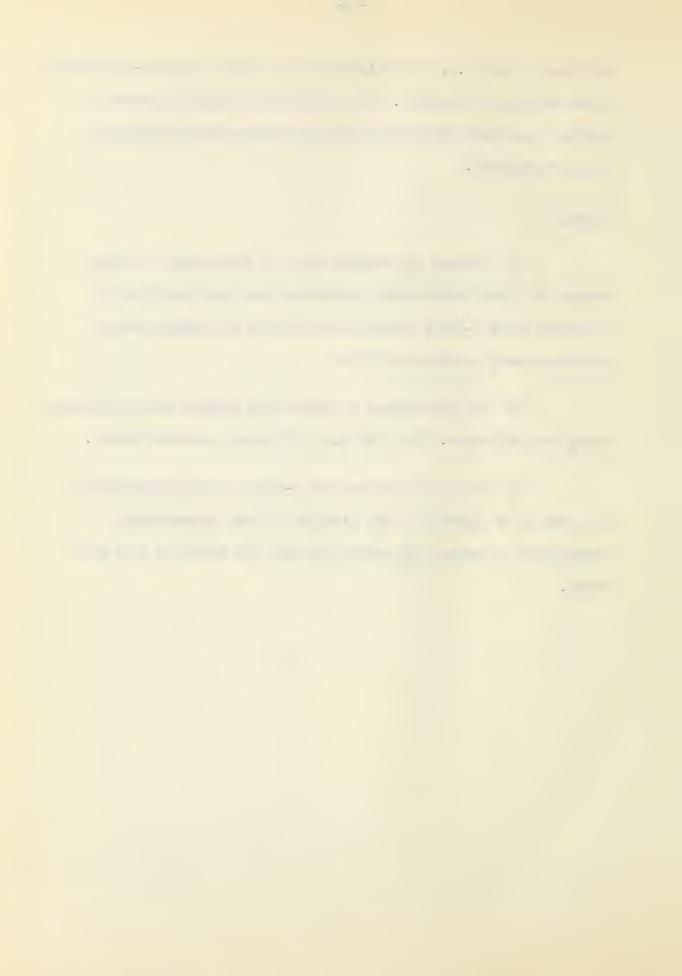
It will be noted in Table 12 that the observed values exceeded the expected values in 7 out of the 10 of the heterozygous genotypes. The means of the total observed and expected classes



differed by only 0.4. This difference was found to be non-significant by an analysis of variance. The analysis did indicate, however, a highly significant difference among the various heterozygous genotypes represented.

Summary

- (1) Average egg weights based on twice weekly weighing during the first three months' production were not significantly different among B-locus genotypes in 1959 but were significantly different among genotypes in 1960.
- (2) The differences in average egg weights were significant among the 1959 sires. The 1960 sire differences were not tested.
- (3) The data indicate that B-locus heterozygous hens do not seem to be superior to the average of their corresponding homozygotes in average egg weight but they are different from each other.



GENERAL SUMMARY

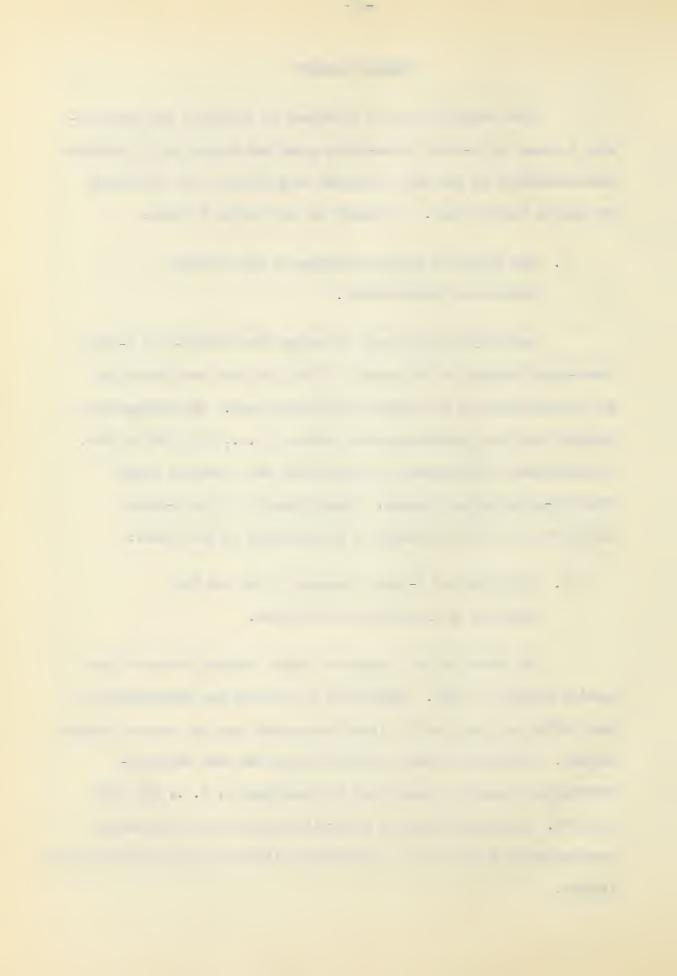
Five experiments were conducted to determine the relationship between the B-locus blood-group genes and economically important characteristics in the White Plymouth Rock flock at the University of Alberta Poultry Farm. A summary of the results follows.

I. The Effect of B-locus Genotype of the Red Cell
Antigens on Hatchability.

Hatchability data were collected from ninety-nine B-locus blood-typed matings in the spring of 1%00 and from one hundred and two typed matings in the summer of the same year. All matings were grouped into heterozygous-expected classes, i.e., 50%, 75% or 100%. No significant differences in hatchability were observed among expected-heterozygous classes. Heterozygosity at the B-locus therefore had little influence on hatchability in this flock.

II. The Effect of B-locus Genotype of the Red Cell
Antigens on Livability to Ten Weeks.

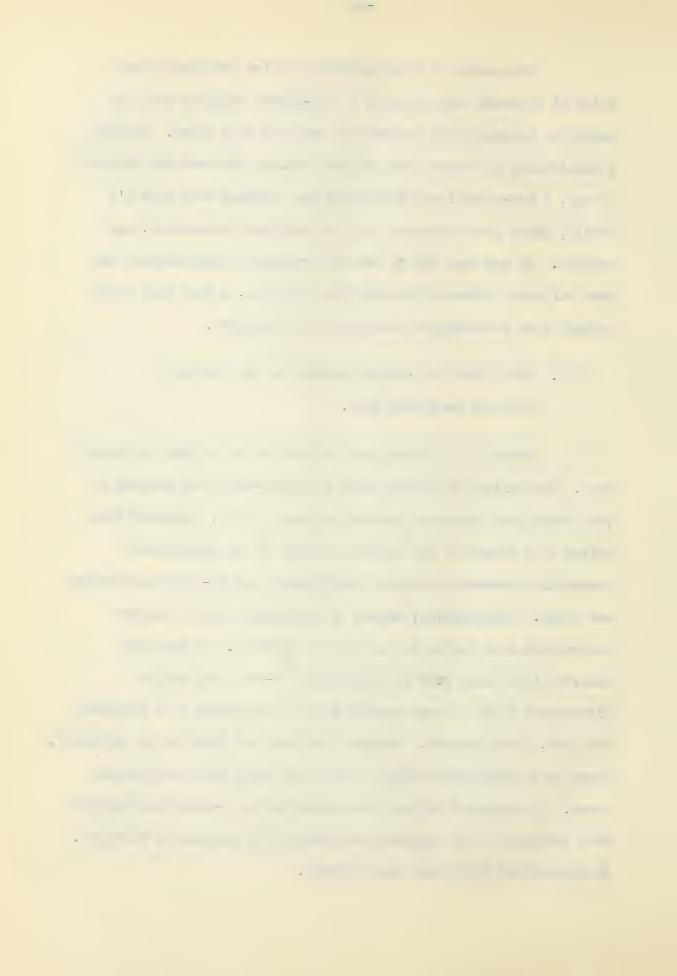
The data for this experiment were obtained from the three special hatches of 1960. Livability to 10 weeks was calculated for each mating as a per cent of fertile eggs and as a per cent of chicks hatched. Livabilities were classified into the same expected-heterozygous classes as mentioned in Experiment I, i.e., 50%, 75% and 100%. Although livability generally improved with increasing heterozygosity there were no significant differences among heterozygous classes.



The number of heterozygotes per sire group which were alive at 10 weeks were compared by chi-square analysis with the number of heterozygotes expected in the same sire group. Although a significant difference was obtained between observed and expected groups, a non-significant difference was obtained when sire 8's family, whose heterozygosity for B alleles was in question, was deleted. In any case six of the nine remaining sire families had more offspring alive at 10 weeks than expected, a fact that might suggest some heterozygous advantage for livability.

III. The Effect of B-locus Genotype of the Red Cell
Antigens on Growth Rate.

Weight at a certain age was used as an estimate of growth rate. The initial study was based on the 10-week body weights of 799 chicks from the three special hatches of 1960. Although these chicks were inbred to the extent of about 7%, no significant correlation between inbreeding coefficients and 10-week body weights was noted. Consequently, degree of inbreeding present was not interpreted as a factor in the results obtained. It was also observed that there were no significant 10-week body weight differences among B-locus genotypes when the weights were adjusted for sire, hatch and sex. Hatches also were not found to be different. There were significant weight differences among sires and between sexes. The observed adjusted mean weights for B-locus heterozygotes were compared to the expected mean weights by analysis of variance. No statistical difference was observed.



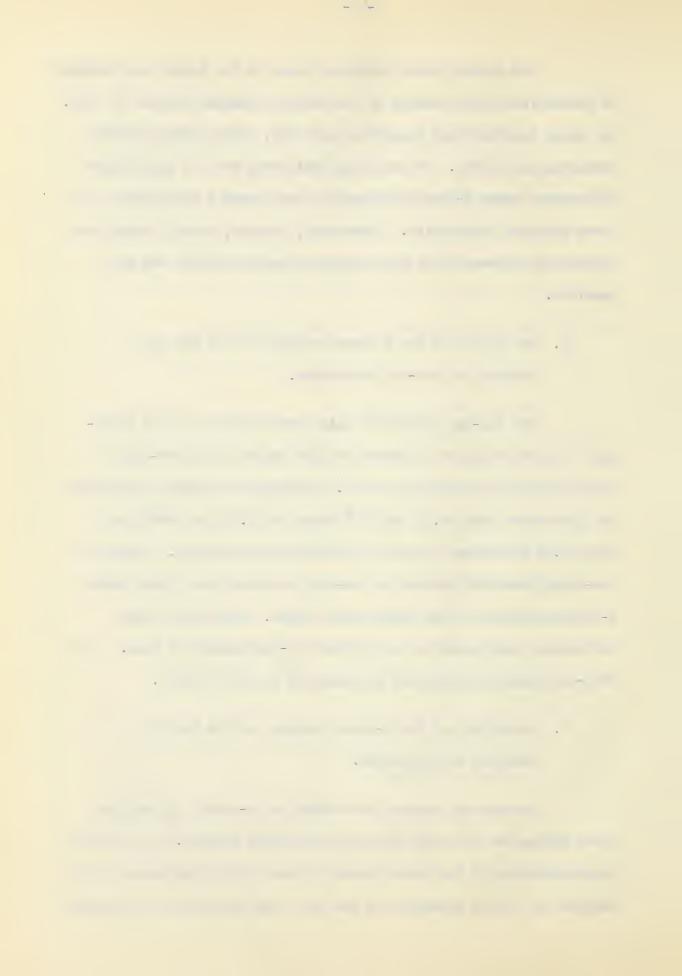
The second growth study was based on the 6-week body weights of chicks from three hatches of the regular pedigree hatches of 1961. All chick families were classified into 50%, 75% or 100% expected-heterozygous classes. It was found that there were no significant differences among B-locus genotypes nor was there a significant sex times genotype interaction. There were, however, highly significant differences between sexes and among dam families within sex and genotype.

IV. The Effect of the B-locus Genotype of the Red Cell
Antigens on Hen-Day Production.

The hen-day production data were collected on 214 blood-typed females which were hatched in 1959 and on 451 blood-typed females which were hatched in 1960. Although the hen-day productions in 1959 varied from 66.5% for Blb5 birds to 77.5% for Blb3 birds, this 11.0% difference was not statistically significant. Similarly non-significant differences in hen-day production were found among B-locus genotypes of the 1960 hatched birds. Significant sire differences were noted in the 1959-60 hen-day production data. The 1960-61 production data were not examined in this respect.

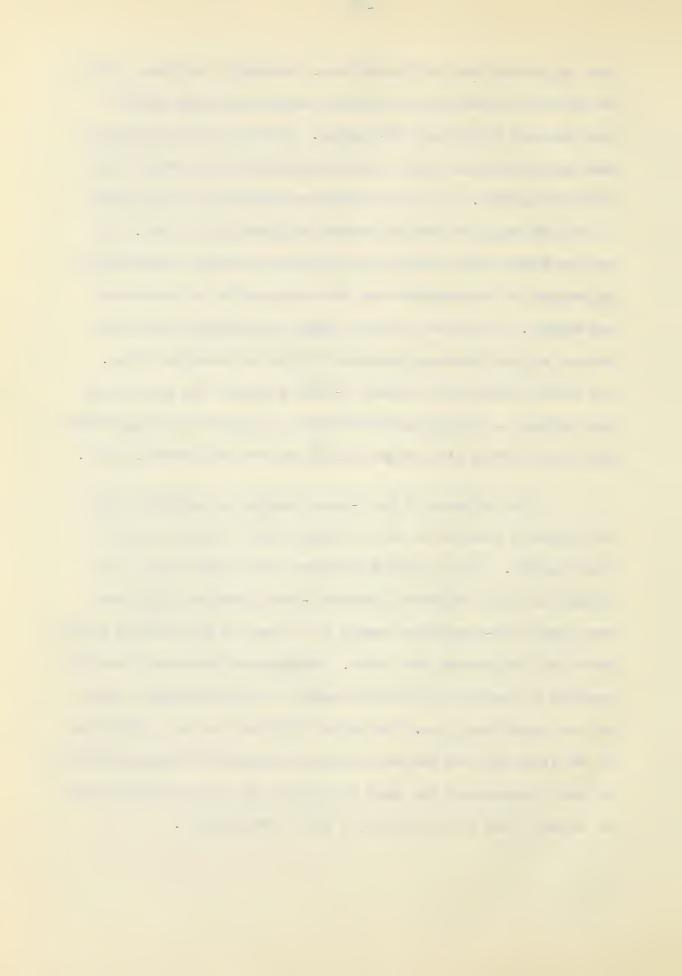
V. The Effect of the B-locus Genotype of the Red Cell
Antigens on Egg Weight.

Average egg weights were based on bi-weekly egg weights taken during the 1959 and 1960 fall production periods. The B-locus blood genotypes of the layers seemed to have little influence on egg weights in 1959 as evidenced by the fact that differences in adjusted



mean egg weights were not significant. Homozygote advantage might be indicated in that the two heaviest adjusted mean egg weights were produced by BlBl and BBB layers. On the other hand, adjusted mean egg weights were highly significantly different among B-locus genotypes in 1960. It is felt that more reliability can be placed on the 1960 egg size data for reasons indicated in the text. It was found that there were no real differences between observed mean egg weights of heterozygotes and the corresponding expected mean egg weights. There were, however, highly significant differences between adjusted mean egg weights of various heterozygous types. The latter suggests that certain B-locus genotypes may possess egg size advantage. Significant differences in adjusted mean egg weights were noted between sire groups in 1959 but were not tested in 1960.

The influence of the B-locus genotype on economic traits was generally found to be less in these studies than that noted by other workers. Sire and dam differences were always found to be significant when considered, whereas B-locus genotype differences were always non-significant except in the case of the 1960 egg weights where real differences were noted. Heterozygous birds were generally superior to homozygous birds with regard to various economic traits but not significantly so. The writer felt that the lower inbreeding of the stock used and the more critical statistical treatment employed in these studies were the chief reasons why the results obtained were at variance with those reported by other investigators.



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APPENDIX A

The methods employed in blood-typing are complicated and perhaps deserving of more detailed explanation than given below. However, since this thesis is concerned mainly with interpretation of results, only brief outlines of the methods used in the actual typing work will be given. For greater detail the reader is referred to Briles et al. (1950).

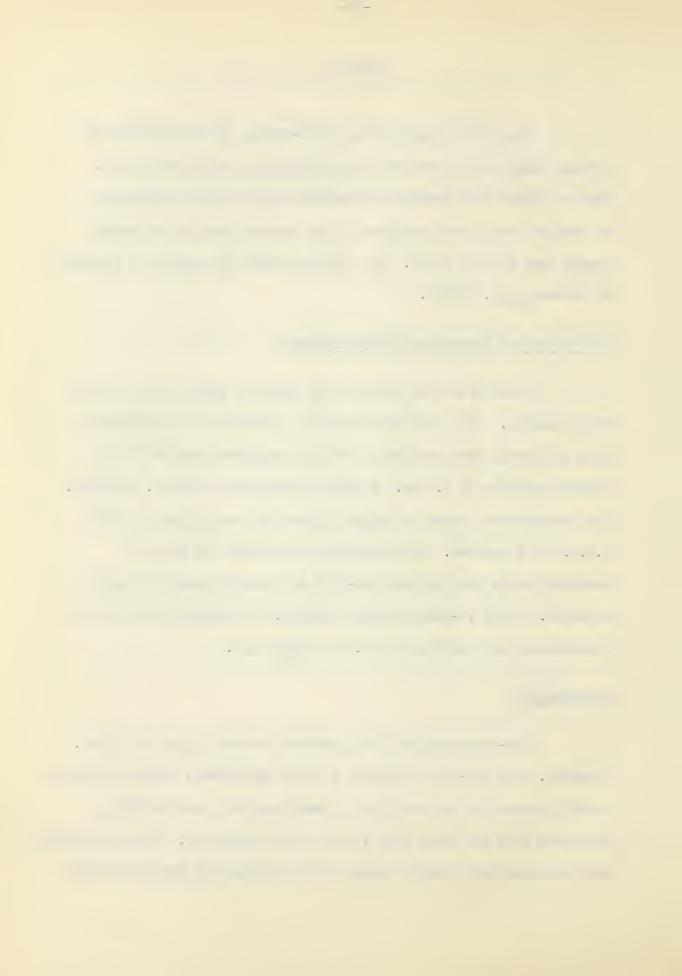
Collection and Processing of Donor Blood

Donor blood was collected by means of heart probe or wing vein bleeding. The blood was mixed with anticoagulent immediately upon withdrawal from the bird. The anticoagulent consisted of a sterile solution of 5.8 gm. of sodium citrate per 1000 ml. of water. The suspension of blood in sodium citrate was centrifuged at 1800 r.p.m. for 2 minutes. The supernatant was drawn off and the remaining cells were diluted two-fold with sterile sodium citrate solution. After re-suspension by shaking, the cells were used for immunization or stored at 38°F. for future use.

Immunization

Iso-immunizations were conducted between close relatives.

Three ml. of a two-fold dilution of cells (see above) from the donors, usually parents of the recipients, were injected, using sterile syringes, into the right wing veins of the recipients. These injections were repeated with freshly prepared cell suspensions every third day



until a strong titer of agglutination was obtained between donor cells and recipient serum.

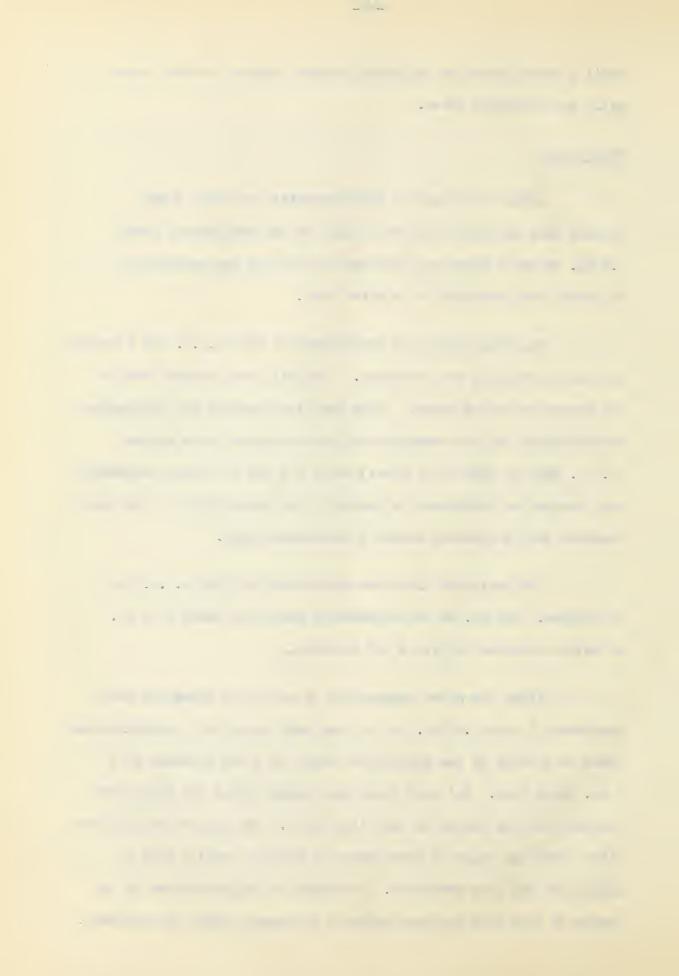
Titer Test

After the second or third injection or when it was thought that antibodies had been built in the recipients, about 2.5 ml. of whole blood was obtained from donors and recipients to permit the conduction of a titer test.

The donor blood was centrifuged at 1800 r.p.m. for 2 minutes and the supernatant was withdrawn. The cells were washed twice in the manner described above. After the final washing the supernatant was withdrawn and the remaining cells were diluted with saline (8.5 g. NaCl in 1000 ml of water) until a 2 per cent cell suspension was obtained as determined by comparing its colour with a 2 per cent standard cell suspension before a microscope light.

The recipient blood was centrifuged at 2000 r.p.m. for 12 minutes. One ml. of the supernatant serum was added to 7 ml. of saline solution to give a 1:8 dilution.

After the above preparation of cells and serum had been completed, 1 drop (.05 ml.) of a 2 per cent donor cell suspension was added to 2 drops of the appropriate serum at a 1:8 dilution in a 3 ml. titer tube. All such tubes were shaken until the cells were removed from the bottom of the titer tubes. The rack which held the titer tubes was shaken 4 times every 15 minutes until 4 sets of agitations had been completed. The degree of agglutination in the bottom of each tube was read before a microscope light and recorded.



Preparation of Antisera

Blood was withdrawn aseptically from the recipients if the donor cells had been agglutinated at a sufficiently high titer by the serum of the respective recipients. Approximately 40 to 50 ml. of blood were taken by heart probe from each bird and mixed with 10 ml. of sodium citrate solution (see above). This blood was then centrifuged at 2200 r.p.m. for 15 minutes. The resulting serum was transferred from the centrifuge tubes into sterile storage bottles where it was cooled for 24 hours and then frozen at -20° F.

Classification of Antisera into Reagents

Prior to the date of the above mentioned work cross-bred birds were obtained by mating Macdonald College White Leghorn males with the University of Alberta White Rock females. The B alleles from this cross were identified by reference reagents purchased from the Texas Agricultural Experiment Station. Certain of these cross-bred birds were sent to Ottawa as a test panel. The sera which were obtained from the University of Alberta White Rock recipients were tested on the cells of this test panel. Each serum sample was classified as a reagent for a specific allele or alleles according to the agglutination patterns observed against the red cells of the test panel. The alleles at the B-locus which were identified at this time were B¹, B², B³, B⁴, B⁵ and B¹².

B-locus Typing

Blood for typing was obtained by wing vein puncture, identified as to source, packed to prevent over-heating or freezing,

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shipped via air to the Animal Diseases Research Institute at Hull, Quebec, where it was B-locus typed using the reagents referred to above.

APPENDIX B

Some of the data which were collected in this study were considered to be non-orthogonal. Thus, a special statistical treatment of part of the data was employed in order to permit inferences about the results.

Analysis of 10-Week Chick Weights

The object of the analysis was to estimate certain parameters from the non-orthogonal data and to make statistical tests on these parameters. The methods followed were the same as those outlined by Henderson (1948), Kempthorne (1952) and Fredeen (1961).

Assuming no interaction a mathematical model was organized as follows:

 $Y_{ijklm} = U + G_i + S_j + H_k + X_l + E_{ijklm}$; where

Yijklm is the 10-week weight of the ijklmth chick;

U is the adjusted population mean;

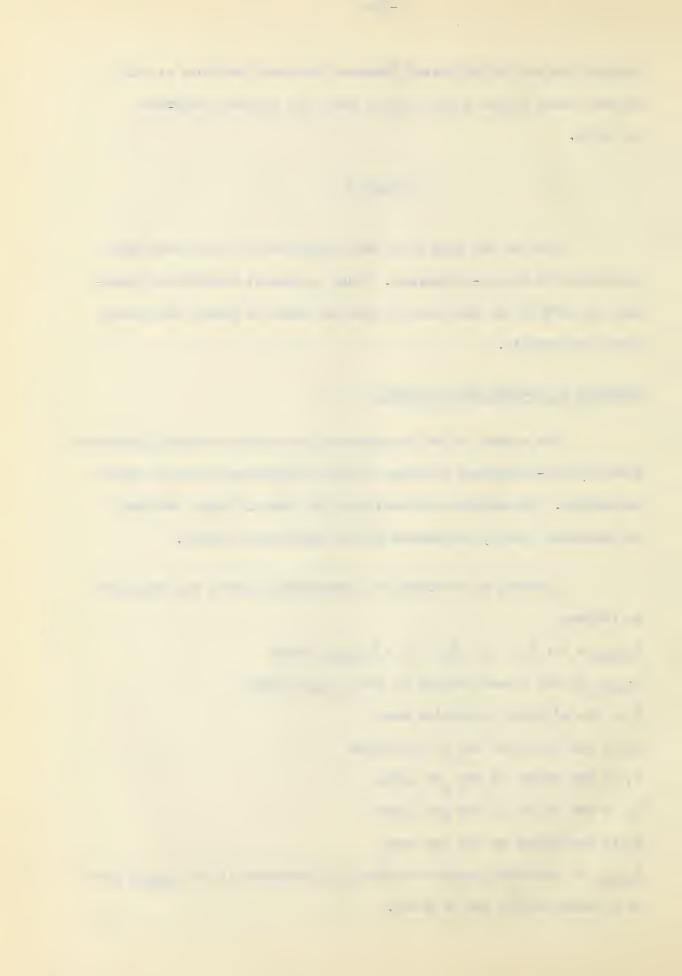
Gi is the effect of the ith genotype;

S; is the effect of the ith sire;

Hk is the effect of the kth hatch;

X1 is the effect of the 1th sex;

E_{ijklm} is an effect peculiar to the mth individual in the ijklth group or a random effect due to error.

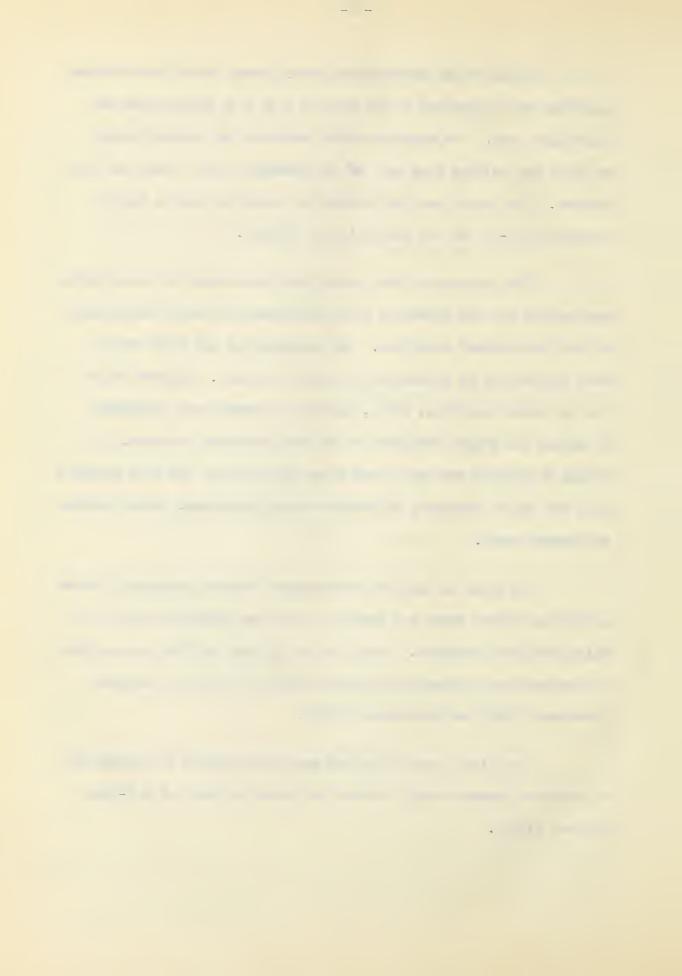


Based on the mathematical model above, thirty simultaneous equations were organized in the form of a 30 x 30 matrix with one right hand side. The equation which contained the largest number of birds was deleted from each of the genotype, sire, hatch and sex classes. The matrix was then solved by inversion using a digital computer (IGP-30) at the University of Alberta.

The solution to the matrix gave an estimate of the adjusted mean weight for the genotype, sire, hatch and sex which corresponded to the four deleted equations. The estimates of all other means were printed out as deviations from this one mean. Adjusted means for all other genotypes, sires, hatches and sexes were calculated by adding the proper deviation to the mean referred to above. It should be pointed out that these means are relative and were intended only for use in comparing differences among genotypes, sires, hatches or between sexes.

In order to test for differences between genotypes a second matrix was solved which was similar to the one described above but which excluded genotypes. Using the two solved matrices, an analysis of variance was conducted utilizing reduction in sums of squares, Henderson (1948) and Kempthorne (1952).

The first inverted matrix was also utilized in testing for differences between sires, hatches and sexes by means of a t-test, Fredeen (1961).



Analysis of Egg Production and Average Egg Weight

The method of analysis of this data was the same as that used above.

The mathematical model was as follows:

 $Y_{ijk} = U + G_i + S_j + E_{ijk}$; where

Y_{i,jk} is the average 3 month egg weight or the hen-day production of the ijkth hen;

U is the adjusted population mean;

Gi is the effect of the ith genotype;

S; is the effect of the ith sire;

E_{ijk} is the effect peculiar to the kth individual in the ijth group or a random effect due to error.

Simultaneous equations were organized according to the above mathematical model. Two matrices were arranged as before for the 1959 and the 1960 production data. Solution of the matrices and statistical treatment was the same as that previously described in the analysis of the 10-week chick weights. In addition, a range test, Duncan (1955), was applied to the adjusted means for genotype of the 1960 egg weight data to determine which means were different from other means.













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